Exploring the role of microbes in coral-algal interactions on the inshore Great Barrier Reef

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December 2019

A thesis submitted for the degree of Master of Philosophy

College of Science and Engineering

ARC Centre of Excellence for Coral Reef Studies

James Cook University

I dedicate this thesis to Tina Cole, Amanda Stonham, Catherine Harper and Zachary Al Moajil-Cole – my unconventional family whom I love unconditionally.

Acknowledgements

Long before Magnetic Island had its current name, it was known as Yunbenun. Hence, I firstly acknowledge the Wulgarukaba and Bindal people, the traditional custodians of the land where data collection and research for this thesis took place. I pay respect to their lores, customs and Elders past, present and emerging.

I extend my gratitude and thanks to my supervisors, David Bourne and Andrew Hoey. Both of you have supported me academically and emotionally, and never gave up on me even when I wanted to give up on myself. Thank you David for allowing me to explore the marine microbial world and investing your time into this research. Thank you Andy for allowing me to share my love of macroalgae with you and lending your ear and advice whenever I needed.

I would also like to thank Hillary Smith, without whom I would be lost. Your guidance in the lab and with bioinformatics has been instrumental in the success of this research project.

The Molecular Ecology and Evolution Laboratory (MEEL) was essential in providing facilities to complete this research. Particularly, I extend my deepest thanks to Carolyn Smith-Keune, the MEEL Manager. You care for the MEEL students even through your own strife, and make our happiness, wellbeing and success your priority, for which I am immensely greatful.

Thank you to the College of Science and Engineering for providing a scholarship and stipend which made this research a reality. Thank you to Jennifer Lappin, Alana Grech, Olga Bazaka, Janet Swanson and Vivian Doherty from the ARC COE Coral Reef Studies for keeping the Centre running and your kind and supportive dispositions. I would also like to thank Jodie Wilson and Lauretta Grasso from the JCU Graduate Research School for continual support and advice throughout my candidature. Thank you to Greg Suossari from JCU Boating and Diving, who supported me throughout all my fieldwork and essentially taught me how to drive a boat!

Many thanks are also extended to Shane Askew from the JCU Advanced Analytical Centre who made the microscopy in this thesis a reality. Further thanks are given to Alison Richards from the JCU Student Equity and Wellbeing services for supporting my mental health throughout my candidature; you were essential in the completion of this thesis.

Two months into my candidature I found out I was unable to continue diving due to medical complications. Hence my sample collection and fieldwork was in the hands of volunteers willing to aid this research. I cannot stress enough how greatful I am to each of you for your work, and putting up with my stress and sea sickness: Chloë Boote, Christopher Hemingson, Hillary Smith, Katie Motson, Katie Peterson, Madalyn Cooper, Paul O'Brien, Samuel Matthews, Sowjanya Chandresekhar and Stephanie Garra.

I would like to thank my friends in the UK for supporting me through my move to Australia. I would not be who I am without any of you. Our friendships have survived regardless of distance and time. You are lifelong family; the sisters I never had. I simply adore each of you: Emma Shillabeer, Isabel Barr, Jenna Dickerson Suzanne Carden-Noad. Thank you to my new friends, who have become a supportive family throughout our time at JCU: Christine Giuliano, Chloë Boote, Donna Simmons, Katie Motson, Katie Sievers, Kelly Hannan, Madalyn Cooper, Michael (Mick) Derrick, Rachel Miller and Taryn Laubenstein.

Thank you to my family for being an example of perseverance and source of endless love. You have supported me through absolutely everything and believed in me to complete this thesis even when I did not believe in myself. Words cannot describe what you mean to me.

Lastly, thank you to Samuel Matthews. Meeting you in Greece 5 years ago was the work of fate, and I would not be here today without you. You have been instrumental in any success I achieve, and throughout the past 5 years and your own PhD made my happiness and success your number 1 priority.

Declaration of the Contribution of Others

The following grants and people assisted in the completion of all thesis chapters.

Research funding

- JCU Postgraduate Research Scholarship and stipend
- Great Barrier Reef Marine Park Authority Science for Management Award
- AIMS@JCU Pilot Research Award
- College of Science and Engineering Student Support Account
- College of Science and Engineering Student Top-up Grant
- College of Science and Engineering Competitive Research Training Grant
- Postgraduate Travel Award – Australian Coral Reef Society
- Earthwatch Recovery of the Reef Grant

Research permits

Permits for sample collection and research were provided by the Great Barrier Reef Marine Park Authority (G15/37574.1 and G16/38348.1)

Thesis committee

Associate Professor David Bourne **–** College of Science and Engineering, James Cook University

Associate Professor Andrew Hoey **–** Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University

Statistical support

Dr. Samuel Matthews

Hillary Smith

Editorial support

Associate Professor David Bourne

Associate Professor Andrew Hoey

Dr. Samuel Matthews

Professor Catherine Harper

Abstract

Macroalgae can rapidly proliferate across areas of degraded reefs preventing coral recovery. Marine microbial communities are fundamental to maintaining reef physiological and ecosystem function. Environmental stress can shift the diversity and resilience of reef-associated microbial communities and these shifts have been shown to exacerbate reef degradation. Describing the response of microbial communities to changes in macroalgae abundance may help identify the contribution of microbes to promoting macroalgae proliferation on degraded reefs. Therefore, this research investigates how coral-algal interactions may influence the diversity and composition of host bacterial communities within macroalgae-dominated environments.

To understand the role of microbes in macroalgae-dominated systems, it is first important to explore baseline microbial communities of common reef macroalgae. Chapter 2 characterises the bacterial community of two *Sargassum* species (*S. aquifolium, S. polycystum*) collected from an inshore fringing reef at Magnetic Island in the central Great Barrier Reef. This site has historically been exposed to poor water quality and high abundances of macroalgae with surveys identifying algal cover at 27.8% and coral cover at 31.7% in the study sites.16S rRNA gene amplicon sequencing was used to profile the microbial communities of the *Sargassum* species and microscopy approaches visualised external surface microbial colonisation. The bacterial community remained consistent between both *Sargassum* species, however differentiation in the bacterial communities of *Sargassum* regions (biofilm, leaf, stem (primary axis), basal growth and holdfast) was observed. In particular, a diverse microbial community was observed on the leaf and biofilm, dominated by bacterial sequences associated with *Bradymonadales, Rhodobacteraceae, Saprospiraceae* and *Loktanella*.

The effect of direct contact between *Sargassum* species (macroalgae) and *Montipora aequituberculata* (coral) on host bacterial communities was also investigated using 16S rRNA gene amplicon sequencing. Samples were collected from Magnetic Island across three proximity treatments (isolation, direct contact and systemic proximity) at three sampling time points (December 2017, February 2018, May 2018) to assess how the host bacterial communities changed in response to direct contact and over seasonal sampling time points. Benthic community surveys revealed the density of *Sargassum* species was higher in February 2018 (43.3% ± 10.0 SE) compared to May 2018 (14.2% ± 2.4 SE). There was a temporal effect from summer to winter on both the *Sargassum* and *M. aequituberculata* associated bacterial communities. For example, sequences affiliated with *Saprospiraceae* (commonly associated with nutrient cycling in macroalgae) in the *Sargassum* bacterial communities were highest in February 2018 compared to May 2018. The reduction in *Saprospiraceae* relative abundance may be linked to the winter senescence of *Sargassum* observed in May, when there is reduced metabolic activity by the host that may result in decreased carbon-rich exudates that drive abundances of *Saprospiraceae*. Overall, while bacterial communities of both *Sargassum* and *M. aequituberculata* were consistent with respect to proximity treatment, a temporal shift in bacteria community structure was observed between winter and summer sampling for both *Sargassum* (December-May, $p=0.013$) and *M. aequituberculata* (February-May, $p=0.003$). The stability of the bacterial communities of *Sargassum* and *M. aequituberculata* when in direct contact with another indicates direct coral-algal interactions appear to have little impact on host microbial community of either adult *M. aequituberculata* or *Sargassum* at Magnetic Island. The stability of the *M. aequituberculata* bacteria community may reflect the tolerance of this species, and explain its success despite the historically high exposure to macroalgae and poor water quality at Magnetic Island.

Despite poor water quality conditions, inshore reefs of the GBR still display diverse coral community assemblages. Future studies should investigate if increases in macroalgae abundance can impact coral-associated microbial communities for a range of coral species with varying sensitivity to environmental stress, including reefs with varying levels of macroalgae abundance. Linking how microbiome changes can impact coral host health using metagenomic and metatranscriptomic approaches is fundamental to confirm the role of microbial communities in maintaining host fitness on reefs with high macroalgae abundances.

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1 Coral-algal interactions and their roles in structuring host microbial communities

1.1 Threats to coral reefs

Coral reefs are highly diverse, complex and productive ecosystems, relying on multipartite networks of biological processes within coral colonies and associated taxa to remain stable and successful (Nyström et al. 2000, Pandolfi et al. 2016, Casey et al. 2017). Reef health across the globe, however, is threatened by multiple environmental and anthropogenic stressors as a result of climate change (Sheppard et al. 2012, Heron et al. 2016, Hughes et al. 2017). However, continued disturbance to coral reefs can create instability within coral environments and increased rates of coral mortality, in turn leading to ecosystem degradation (Heron et al. 2016).

While cycles of disturbance and recovery are natural on coral reefs, there is concern that the increased frequency and severity of disturbances has hindered the ability of coral reefs to recover (Dollar and Tribble 1993, Fox 2011, Hughes et al. 2018). The Great Barrier Reef (GBR), for example, lost approximately half of its live coral cover between 1985 and 2012 (De'ath et al. 2012), and has since experienced additional coral mortality as a result of cyclones, crown of thorns starfish outbreaks (Matthews et al. 2019, Mellin et al. 2019) and two severe coral bleaching events (Hughes et al. 2017, 2018). The increasing severity and frequency of disturbances to the GBR are postulated to make it near impossible for reefs to recover to previous configurations and instead, the maintenance of biological functions is the emerging goal of coral reef conservation and management (Hughes et al. 2017).

With reduced capacity to cope with multiple stressors, disturbances to reefs can clear large areas, making them vulnerable to the establishment of other organisms where coral ecosystems were once dominant. Macroalgae are often quick to establish in spaces created through continued decline in coral health (Hughes et al. 2007, Mumby 2009, Dubinsky and Stambler 2011). Though macroalgae are key components of coral reef ecosystems, providing food for higher trophic organisms and nurseries for juvenile fish (Lachnit et al. 2011, Martin et al. 2014), they can be of concern to reef health when they proliferate and overgrow previously coral-dominated areas (McCook et al. 2001, Idjadi et al. 2006, Rasher et al. 2012). Coastal and inshore reefs are at higher risk of macroalgae overgrowth as a result of their proximity to additional anthropogenic stressors such as increased sediment and nutrient loading (Ayling and Ayling 2005, Fabricius 2005, Fabricius et al. 2005). High nutrients, turbidity, and naturally lower herbivory rates on inshore reefs have been linked in many ecosystems to increasing macroalgae cover and resultant decreasing coral cover and diversity (Wismer et al. 2009, Cheal et al. 2010, Brodie et al. 2012). Furthermore, exposure to chronic stressors can then exacerbate the effects of acute disturbances, hindering coral reef recovery, and increasing likelihood of macroalgae establishing on coral reefs (De'ath et al. 2010, Graham et al. 2011).

Coral colony health and fitness can be negatively impacted by increased macroalgae abundance, for example by shading and abrasaion (Nugues et al. 2004, Morrow et al. 2011). Such impacts may arise via direct mechanisms such as competition for space, which can inhibit coral larvae recruitment and prevent coral propagation post-disturbance (Kuffner et al. 2006, Birrell et al. 2008a, Webster et al. 2015, Clements et al. 2018). Additionally, indirect processes resulting from abundant macroalgae may also reduce coral health, such as allelopathy (Morrow et al. 2011) and the release of excess dissolved organic carbon, which can induce coral mortality by fueling microbial activity (Smith et al. 2006, Haas et al. 2016). In some degraded reef systems, where a combination of poor water quality, reduction in herbivorous fish biomass

and space clearing events (e.g. tropical storms, mass coral bleaching, coral disease) occur, shifts from coral to macroalgae-dominated systems have been observed (Idjadi et al. 2006, Hughes et al. 2007). Return of coral dominance is rare once newly dominant fleshy macroalgae communities have established (Mumby 2009, Sheppard et al. 2012, Holling 2016), and Hughes (1994) noted that in many cases of macroalgae regime shifts, recovery back to coral-dominated reefs may not in fact be possible.

Triggers of shifts from coral to macroalgae dominance have been investigated with three main processes being identified:

1) reduced herbivory from removal of herbivorous organisms with the potential to control macroalgae populations (Box and Mumby 2007, Mantyka and Bellwood 2007, Hoey and Bellwood 2011),
2) nutrient and sediment loading from coastal development (Smith et al. 1981, Fabricius 2005),
3) space clearing through bleaching and storm events (Dubinsky and Stambler 2011, Hughes et al. 2018).

Whilst various ecological triggers can be attributed to prompting macroalgae dominance on coral reefs, there are different circumstances under which macroalgae dominance can persist on coral reefs. For example, reefs in the Caribbean experienced outbreaks of coral disease (Bythell and Sheppard 1993), severe tropical storms (Bythell et al. 1993), coral bleaching (Kramer et al. 2003), reduction of grazer controls of macroalgae after the die-off of the *Diadema antillarum* urchin (Lessios et al. 1984), overfishing of herbivorous fishes (Hughes 1994), and a reduction in water quality (Littler et al. 1993). These factors worked synergistically to clear space and provide more favourable conditions for macroalgae species (Bellwood et al. 2004, Mumby 2009). These pre-conditions are not always necessary, however,

for example within the Great Barrier Reef Marine Park, shifts to high macroalgae biomass were recorded in 'no take' areas where herbivorous fish, while low in diversity, were not harvested and where nutrient levels were not unusually high (Cheal et al. 2010).

Currently, it appears that very little can be done to reverse macroalgae dominant reefs back to coral-dominated environments, particularly on a larger scale. Direct removal of macroalgae to clear space for coral recruitment has been proposed (Ceccarelli et al. 2018), but on reefs where macroalgae is established this action fails to target the underlying mechanisms thought to sustain algal dominance. Uncovering the systems and mechanisms that maintain a downward trajectory into a macroalgae regime may help inform development of tools and techniques to effectively manage macroalgae regime shifted reefs. Current attempts to understand the mechanisms that drive macroalgae dominance have been limited to explaining only why such states occur (ecological tipping points), but there has been increased interest in the role of microbial processes additionally contributing to the mechanisms of macroalgal persistence in degraded coral environments (Figure 1).

As the global state of coral reefs continues to decline at an alarming rate, the risk of losing coral reefs to macroalgae-dominated environments has never been higher. Understanding mechanisms that may sustain macroalgae dominance can aid restoration attempts, as we are then able to address the underlying processes that contributed to high macroalgae abundance (Figure 1.1). Therefore, it is important to consider the potential role of microbes in reinforcing macroalgae abundance on coral reefs.

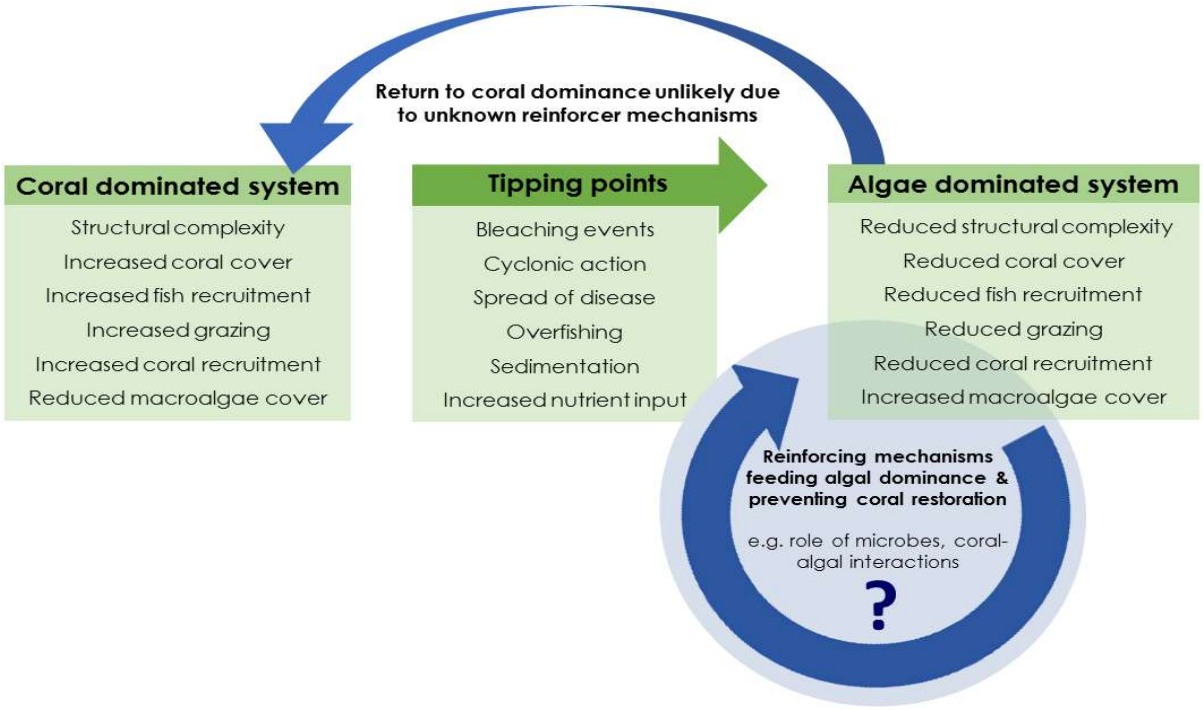


Figure 1.1 'Situating the review'. Green colouring represents established ecological processes and conditions contributing to shifts from coral to macroalgae dominance. Blue colouring represents unknown reinforcing mechanisms contributing to sustain macroalgae dominance. Investigating these mechanisms is essential to unlocked why macroalgae-shifted environments persist and help inform coral restoration strategies. This diagram highlights the knowledge gaps which this review will focus on (information highlighted in blue).

1.2 Microbes in coral reef environments

Marine microbes are associated with a wide variety of organisms, and have become increasingly recognised as essential contributors in maintaining host metabolism and fundamental function (Taylor et al. 2007, Webster and Thomas 2016). Microbes form the foundation of primary production in marine environments, efficiently assimilating limiting nutrients such as nitrogen and carbon to make them accessible for use by higher trophic levels (Azam and Malfatti 2007, Thornton 2012).

Microbes in coral reef environments exist in complex systems of free-living and host-associated communities, linked to benthic and pelagic environments. Marine biofilms are formed from the excretion of extracellular polymeric substances (EPS) (Underwood and

Paterson 2003) laid down by a variety of bacteria and diatoms (Patil and Anil 2005). Biofilms can form niches of marine microbiomes and form on a variety of surfaces (e.g. macroalgae), acting as a habitat for pelagic microorganisms and a food source for higher trophic levels such as deitivores (Eich et al. 2015). Microbial communities in seawater are diverse and their community structure is dependent on complex interactions between the water nutrient profiles and physical environmental parameters (Azam 1998, Stocker 2012). Pelagic microbial communities can be influenced by environmental change, with recent research highlighting that the seawater microbiome can act as an indicator for changes in the reef environment (Glasl et al. 2019). Microbial communities in coral reefs have also been associated with a range of organisms making up the coral benthos. Sponges, for example, form diverse relationships with microbial communities linked with water filtration and host physiological function (Hentschel et al. 2012, Webster and Taylor 2012, Webster and Thomas 2016). Similarly, microbial communities establish close symbiotic relationships with corals and have been implicated as critical in maintaining coral health and resilience (Ducklow and Mitchell 1979, Harris et al. 2001). The best known example is the photosynthetic dinoflagellate (Symbiodinaceae) that resides within the coral gastrodermal layer and provides essential energy to the coral (Falkowski et al. 1984, Rosenberg et al. 2007). In addition to the Symbiodiniaceae, bacteria belonging to the genus *Endozoicomonas* have been regularly discovered in association with many coral genera and other marine invertebrates (Bayer et al. 2013, Neave et al. 2016, Pogoreutz et al. 2018). Genomic studies on *Endozoicomonas* spp. have highlighted the potential for its important contribution to holobiont important function, such as transport of organic molecules and the synthesis of amino acids (Neave et al. 2017).

Due to the close association between coral hosts and their microbial communities, the term ‘coral microbiome’ is used to refer to the diverse and abundant prokaryotes and eukaryotes (bacteria, fungi, archaea, protists and viruses) that make up a species-specific internal microbial

community, as distinct from surrounding seawater (Ritchie 1997, Harris et al. 2001, Rohwer et al. 2002, Bourne and Munn 2005, Sunagawa et al. 2010). A stable microbiome supports a variety of functions to maintain host health and productivity, through for example production of antibiotics to deter opportunistic pathogens (Nissimov et al. 2009), and biogeochemical cycling of nitrogen, carbon and sulphur (Raina et al. 2009, Kimes et al. 2010).

More recently, the 'macroalgae microbiome' has been recognised and may be integral in maintaining macroalgae health. Barott et al. (2011) revealed that tropical macroalgae functional groups in the Caribbean have diverse and significantly different microbial communities from each other and their surrounding environment. For example, crustose coralline algae (CCA) were dominated by sequences closely related to *Cyanobacteria*, *Lactobacillus* and *Chloroflecaceae* while *Dictyota bartayresiana* was dominated by sequence groups related to *Cyanobacteria* and unknown *Bacteria*; *Halimeda opuntia* (green seaweed) was dominated by sequences relating to *Cyanobacteria* Group I, *Lactobacillus* and *Rhodobacteraceae*; and turf algae was dominated by sequences related to *Acidovorax, Lactobacillus* and *Cloacibacterium*.

Egan et al. (2013) highlighted the importance of macroalgal-bacterial interactions in maintaining algal health, and emphasised that the 'seaweed holobiont' associated with macroalgal biofilms is just as important as the coral holobiont in promoting reproduction, colonisation, and protection against fouling organisms. It has proven difficult, however, to explore consistency in macroalgae host microbiomes. Where stable host microbial communities have been shown in some cases (for example, in the entophytic communities of *Caulerpa* sp. and *Bryopsis* sp. (Hollants et al. 2011)), it has been difficult to demonstrate in others. Extensive 16S rRNA sequencing of the green seaweed *Ulva australis*, for example, was unable to detect a species-specific core community, with only 6 of 528 detected bacterial species being consistent across 6 individuals (Burke et al. 2011a). Nonetheless, further shotgun

metagenomic sequencing of *U. australis* showed the host community may be dictated by function rather than microbial taxonomy (Burke et al. 2011b).

Microorganisms respond rapidly to altered environmental conditions in part due to short generational turnaround times (Torda et al. 2017). Environmental stress can disturb the stability of host-associated microbial communities (i.e. dysbiosis) which subsequently can impact host fitness (Schimel etl al. 2007, Egan et al. 2013, Bourne et al. 2016, Rocca et al. 2019). Zaneveld et al. (2017) proposed the 'Anna Karenina principal' whereby dysbiotic individuals vary more in microbial composition compared to healthy individuals, which has been observed within coral microbiomes experiencing environmental stress. Shifts in coral bacterial communities have been observed throughout periods of bleaching events (Bourne 2008), with exposure to above average temperature (Zaneveld et al. 2016), and corals displaying disease lesions have more variable microbial communities compared to stable microbiomes of healthy corals (Thurber et al. 2017, Maher et al. 2019).

As shifts in microbial communities occur within corals during periods of environmental stress, it is also important to consider if there are connections between microbial and ecosystem stability from cumulative environmental stressors. Bourne et al. (2016) highlighted that with continued environmental stress, both the coral microbial communities and broader coral reef ecosystem shift into a new state of reduced diversity and resilience. More broadly, ecosystems and microbial communities have been shown to co-vary in response to environmental stressors (Reshef et al. 2006, Bordenstein and Theis 2015). However, there is currently limited understanding of how microbes contribute to changed ecosystem structure and function. As coral reefs continue to degrade and the risk of shifts from coral-dominated to macroalgae-dominated systems increases, it is vital to consider how microbes may play a mechanistic role in macroalgae regime shifts.

1.3 Microbial processes facilitating macroalgae abundance

Microbes potentially reinforce macroalgae regimes through a number of complex pathways. One of the most widely recognised ways is allelopathy (Gross 2003, Morrow et al. 2011, Rasher et al. 2011). Allelochemicals are energetically costly for macroalgae to produce, but can act as an anti-herbivory defence mechanism (Baumgartner et al. 2009) and inhibit the growth of pathogenic and fouling organisms (Puglisi et al. 2007). Furthermore, allelopathy through direct contact can cause necrosis of coral tissue, creating localised space for macroalgae to continue to spread (Rasher and Hay 2010). Higher macroalgae abundance can increase the concentration of algae-derived chemical exudates in the water column, creating further pathways by which algal exudates degrade corals and aid macroalgae proliferation (Barott et al. 2011, Egan et al. 2013).

Smith et al. (2006) established that exudates released into the water column can cause indirect coral mortality. To demonstrate this, *Pocillopora verrucosa* and *Dictyosphaeria cavernosa* were kept in a tank and separated by a 0.02 μm filter to allow any chemical exudates released by the algae to pass through. In all instances, the coral suffered 100% mortality within two days. Physiological measurements (for example, tissue necrosis from hypoxia) showed an increase in direct coral stress with closer proximity between coral and algae due to higher microbial activity in the interface between them. The role of allelopathy, however, in promoting macroalgae growth is localised and often species-specific (Vieira et al. 2016b).

Microbialisation and the DDAM model (Dissolved organic material, Disease, Algae and Microbes) are predicted to influence microbial metabolic pathways and nutrient dynamics in coral reef ecosystems (Roach et al. 2017). Microbialisation represents a quantitative metric for coral reefs, directly referring to observed shifts in trophic structure, making macroalgae and microbes competitively superior over corals (Haas et al. 2016, Roach et al. 2017). Algal

overgrowth releases excess photosynthate into the environment (Carlson et al. 2002), which in excess cannot be efficiently assimilated by higher trophic organisms such as corals (Dinsdale and Rohwer 2011). Surplus of photosynthates derived from macroalgae is more efficiently assimilated by microbial communities with metabolic pathways shifting from predominantly autotrophic to copiotrophic and heterotrophic (Dinsdale and Rohwer 2011, Haas et al. 2016, Roach et al. 2017). These shifts deprive corals of available nutrients and oxygen, and microbes outcompe corals for the essential primary productivity they depend on for survival (McDole Somera et al. 2016). Ultimately, this transfer can cause localised hypoxia, stunted coral growth, and reduced reproductive capacity of corals (Haas et al. 2016). The microbialisation process can act in combination with the stressors, initially triggering macroalgae dominance. Haas et al. (2016) linked microbialisation and the DDAM model, implying both act in conjunction with the other as a mechanism to reinforce macroalgae abundance. The DDAM model (Figure 1.2) links increased abundance of macroalgae to (1) increased release of macroalgae derived organic compounds, (2) promotion of opportunistic pathogen invasion, and (3) stimulation of coral mortality (Barott et al. 2011, 2012). The model is a feedback loop where the processes already associated with increased macroalgae, facilitate additional algal biomass and coral decline (Smith et al. 2006).

Microbialisation and the DDAM model suggest changes in microbial activity can contribute to increased macroalgae growth in coral reef ecosystems. Furthermore, the models indicate environmental change can enhance macroalgae associated microbes in such a way as to benefit macroalgae proliferation. Microbialisation of reefs and the DDAM model work indirectly alongside ecological tipping points, rather than contributing to direct coral-algal interactions. Changes to host microbiomes due to environmental change and/or contact with other organisms can alter the stability and ultimately health of organisms within an ecosystem. Therefore it is important to consider the role of direct coral-algal microbiome interactions alongside indirect

pathways to fully understand the ecosystem processes that may reinforce macroalgae populations.

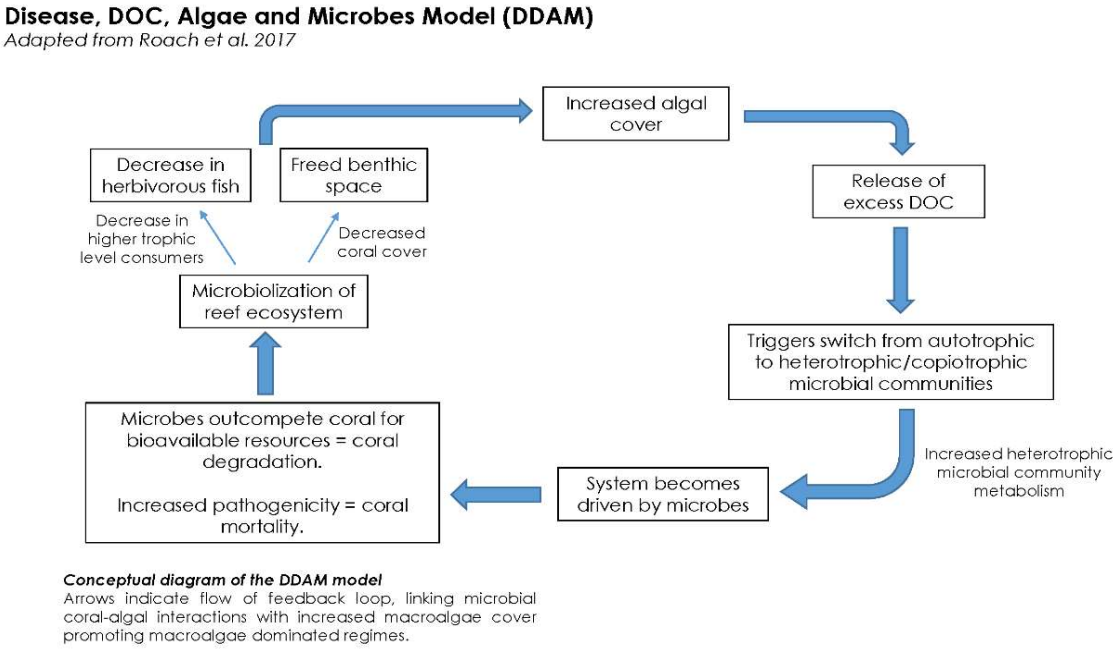


Figure 1.2 Increased benthic macroalgae cover can cause the release of excess bioavailable DOC, triggering a switch from autotrophic to copiotrophic/heterotrophic microbes. This can initiate microbialisation of the reef ecosystem. Changes in microbial community structure can lead to coral degradation, as the microbes outcompete coral for bioavailable resources. This deprives corals of the necessary nutrients they need for survival, leading to a higher chance of coral degradation. Furthermore, degraded reefs are more susceptible to opportunistic pathogens and therefore coral mortality from disease. Microbialisation of reef ecosystems encourages bottom-up trophic control, leading to decreased numbers of herbivorous fish. Freed benthic space (i.e. benthic space available to colonise) coral degradation and less grazing pressure yields a positive feedback loop promoting coral mortality and macroalgae-dominated regimes.

Pratte et al. (2018) investigated direct microbiome interactions between turf algae and coral, showing that the coral microbiome becomes similar to the turf microbiome in areas of direct contact. These results suggest that the coral microbiome is vulnerable to microbial colonisation from turf microbes, but not vice versa. Thus, algal turf microbiome stability and coral microbiome vulnerability at areas of direct contact may contribute to loss of coral and proliferation of macroalgae. The ecological tipping points of macroalgae regimes have been established (e.g. reduced herbivory, nutrient and sediment loading, space clearing), though

currently our understanding of how direct interaction-induced shifts in microbial communities associated with both coral and macroalgae impacts host fitness is poorly understood. Barott et al. (2012) proposed a model whereby some fleshy macroalgae can act on a micro-scale to stress corals, leading to macro-scale changes in reef ecology. We hypothesise that such micro- to macro- scale interactions can exacerbate coral decline across macroalgae-dominated environments and create space for macroalgae to persist.

1.4 Study site and species

Research for this thesis was undertaken on the inshore fringing reefs of Magnetic Island, situated off the coast of Townsville in the central GBR. Magnetic Island acts as an ideal study site to research the role of microbes in coral-algal interactions due to the high abundance of macroalgae on these reef environments.

Magnetic Island's fringing reefs are still host to diverse coral assemblages including *Acropora* spp., *Monitpora* spp., *Porites* spp. and *Favia* spp. (Marshall and Baird 2000, Bourne 2005, Glasl et al. 2019, Saha et al. 2019), and have displayed resilience in response to bleaching and cyclone events (Ayling and Ayling 2005). That said, over the last 30 years, coral cover *around* Magnetic Island has declined from ~40-50% to ~20-25% (Thompson et al. 2017). Long-term data records, highlight that coral cover on Magnetic Island at a depth of ~5m has stabilised to ~20% for the past 15 years, with coral condition nevertheless characterised as 'poor' to 'very poor' (Thompson et al. 2017). Furthermore, water quality at the site has been described as 'poor' on the basis of high turbidity levels and elevated chlorophyll-*a* and phosphorus concentrations (Schaffelke et al. 2012).

Over the last 30 years, reefs around Magnetic Island have been subjected to a steady increase in macroalgae abundance. This can be correlated to high turbidity discharged from the Burdekin River and maintenance dredging of the Townsville port shipping channel (Bak 1978,

Browne et al. 2013a). Mapstone et al. (1992) recorded numerous macroalgae genera on Magnetic Island including: *Caulerpa*, *Ceratodictyon*, *Halimeda*, *Lobophora*, *Padina* and *Sargassum*. Their study showed that *Sargassum* species were the dominant taxon, contributing to over 85% of the macroalgae biomass across the island (Mapstone et al. 1992). *Sargassum* species are a brown, canopy-forming fleshy macroalgae with numerous species showing it to be distributed from arctic to tropical climates (McCourt 1984). Across coral reefs, *Sargassum* species grow sub-tidally and undergo seasonal cycles of growth and senescence (Martin-Smith 1993). Summer growth and abundance of *Sargassum* species can blanket inshore reefs, with winter senescence starting after the algae has reproduced (Martin-Smith 1992). *Sargassum* species rarely completely senesces on reefs, however and, where environmental stressors are chronic and persistent, the reef can be left dominated by *Sargassum* species year round (Ceccarelli et al. 2018). *Sargassum* is an ideal study species to explore microbial coral-algal interactions as there is a knowledge gap regarding the effect of microbes from fleshy macroalgae on coral. Furthermore, the microbiome of tropical species of the algae is unknown, providing a platform from which to explore the microbial community of one of the most abundance macroalgae on the GBR. Moreover, the natural growth and senescence cycle of the alga acts as a proxy for 'regime shift'; investigating coral-algal interactions from high to low abundance of macroalgae. *Montipora aequituberculata* was selected as our coral species as it is abundant across the reefs of Magnetic Island, and the response of the microbial community of the coral to environmental shifts is unknown.

1.5 Thesis outline

The overarching aim of this thesis is to examine whether direct coral-algal interactions have the potential to influence coral and macroalgae associated microbiomes on the inshore Great Barrier Reef. Ecological relationships between coral and macroalgae have been well documented, resulting in extensive knowledge regarding ecological competition between the

two organisms. Research exploring microbial communities in marine environments is developing rapidly, with many studies documenting the microbes associated with marine organisms and their response to stress. Whilst microbial responses to stressors such as bleaching and ocean acidification have garnered attention, there has been little development of microbial responses to increasing macroalgae abundance on coral reefs. Current attempts to understand underlying mechanisms that may drive macroalgae persistence have not fully explained why such states are maintained and therefore microbes likely play some role in facilitating the outcome of coral-algal interactions. Thus, this thesis investigates the baseline microbial communities of *Sargassum* species and *M. aequituberculata* to understand how increasing macroalgae abundance influences both the coral and macroalgae microbiomes.

Whilst this thesis aims to explore microbial coral-algal interactions, it is imperative first to characterise microbes associated with tropical macroalgae. Thus, Chapter 2 of this thesis characterises the bacteria community of two *Sargassum* species (*S. aquifolium* and *S. polycystum*). These communities were investigated used 16S rRNA gene amplification and microscopy (light microscopy, scanning electron microscopy and fluorescent *in situ* hybridisation). The *Sargassum* samples were separated into different morphological components to detect microbially diverse areas of the alga and potentially influential morphological components in coral-algal interactions. Chapter 2 therefore provides a baseline understanding of the *Sargassum* species microbiome.

Chapter 3 investigates the interactions between the dominant algal species *Sargassum* and coral *M. aequituberculata* on reefs at Magnetic Island. This study took place across three sampling time periods from the beginning of the austral summer to winter to investigate if *Sargassum* growth and senescence impacts both the algae and coral microbiome. This study also investigates direct coral-algal interactions by exploring the effect of 'proximity' between coral

and macroalgae. *Sargassum* species and *M. aequituberculata* samples were collected in isolation, direct contact and systemic contact (10cm away from point of direct contact) to examine whether direct contact between organisms shifts the bacterial communities of *Sargassum* and/or *M. aequituberculata*. Chapter 3 also used 16S rRNA gene amplification, as well as collecting descriptive ecological data to examine how the surrounding ecology changed throughout the sampling time period. This chapter provides insight into how the *Sargassum* species and *M. aequituberculata* bacterial communities change over time and with direct contact interaction, acting as a starting point to explore how microbial coral-algal interactions may influence coral and macroalgal health.

Chapter 4 is a general discussion, providing a synthesis of the major findings of the thesis. This chapter evaluates the significance of this research and highlights key further research to be undertaken to improve understanding of the role of coral-algal interactions in contributing to coral and macroalgal health.

2 Exploring the tropical *Sargassum* macroalgae microbial community

2.1 Abstract

Disturbed coral reef systems are vulnerable to shifts from coral to macroalgae-dominated environments, which can also shift reef microbiome diversity and function. To understand the role of microbes in macroalgae-dominated systems, it is important to explore baseline microbial communities of common reef macroalgae. This study describes the bacterial community associated with two *Sargassum* species (*S. polycystum* and *S. aquifolium*) collected from Magnetic Island in the central Great Barrier Reef (GBR). *Sargassum* samples were dissected into 5 regions (biofilm, leaf, stem (primary axis), basal growth and holdfast) to investigate if the bacterial community, assessed through 16S rRNA gene amplicon profiling, differed across various *Sargassum* regions. Whilst *S. aquifolium* and *S. polycystum* microbial communities were not significantly different, differentiation between the upper (biofilm and leaf) and lower (holdfast and basal growth) regions was observed. The biofilm and leaf were dominated by bacteria potentially involved in nutrient cycling (*Saprospiraceae, Loktanella, Bradymonadales*), whilst the holdfast and basal growth were dominated by bacteria associated with marine sediments and anaerobic respiration (*Firmicutes, Geobacter*). Scanning electron microscopy (SEM) and fluorescent *in situ* hybridisation (FISH) visualised structures on the *Sargassum* leaf biofilm. Numerous diatoms associated with the formation of marine biofilm were observed, alongside coccoid and filamentous bacteria-like structures. This study reveals *Sargassum* species have a diverse bacterial community that differs across regions that may underpin host fitness.

2.2 Introduction

With continued decline of coral reef health globally (Pandolfi et al. 2016, Hughes et al. 2017), there has been increased concern regarding shifts from coral-dominated to macroalgae-dominated systems (McCook et al. 2001, Ceccarelli et al. 2018). Increased occurrence of extreme climatic events (e.g. mass bleaching) (Hughes et al. 2018), declining water quality from coastal development (De'ath et al. 2010) and coral predator outbreaks (Pratchett et al. 2017) can reduce coral cover. Commonly, opportunistic macroalgae rapidly proliferate across areas of degraded reef (Rasher et al. 2012, Sheppard et al. 2012), and rapidly colonise dead coral skeletons (Diaz-Pulido and McCook 2003, Leggat et al. 2019), which can occupy space and hamper coral recovery (Hughes et al. 2007, Webster et al. 2015, Graham et al. 2015, Morrow et al. 2017). Return of coral dominance is rare once macroalgae communities have established (Graham et al. 2015). As such, there has been interest in understanding the pathways by which macroalgae persist on coral reefs, with current research showing these pathways as often complex and multifaceted mechanisms (Mumby 2009, Brown et al. 2018, Ceccarelli et al. 2018). While the role of microbes in coral-algal interactions is poorly understood, it is acknowledged that it may additionally contribute to macroalgal persistence in degraded coral reef environments (Haas et al. 2016).

Microbial communities are increasingly recognised as performing an important role in maintaining tropical marine ecosystem function (Kimes et al. 2010, Glasl et al. 2016). Microbes form the foundation of primary productivity in coral reef environments, efficiently assimilating limiting nutrients (e.g. nitrogen, carbon and phosphorus) to make them accessible for use by higher trophic organisms (Falkowski et al. 2008, Raina et al. 2009). Microbial communities play a further active part in maintaining coral health and resilience (Ainsworth et al. 2010, Bourne et al. 2016) which has led to use of the term 'coral microbiome', which refers to the diverse and abundant prokaryotes and eukaryotes (bacteria, fungi, archaea, protists and viruses)

that make up a species-specific microbial community, distinct from surrounding seawater (Rohwer et al. 2002, Reshef et al. 2006). A stable microbiome supports a variety of functions to maintain host health and productivity, for example, production of antibiotics to deter opportunistic pathogens and biogeochemical cycling of nutrients internally in hosts (Reshef et al. 2006, Rosenberg et al. 2007, Nissimov et al. 2009). Shifts in host microbial community composition can occur, however, during periods of reef stress, such as disease and lower pH (Bourne et al. 2009, Thurber and Willner-Hall 2009, Meron et al. 2011). Microorganisms respond rapidly to altered environmental conditions in part due to short generational turnaround, and thus microbial communities can become easily destabilised (dysbiosis), influencing host fitness (Schimel et al. 2007, Egan et al. 2013, Bourne et al. 2016, Rocca et al. 2019).

Similar to the coral microbiome recent studies have promoted the concept of the 'macroalgae microbiome' with the associated microbial communities having some role in underpinning host fitness. Egan et al. (2013) highlighted the importance of macroalgal-bacterial interactions in maintaining algal health; epiphytic bacterial communities assist in normal morphological development, and bacteria with anti-fouling properties to defend against pathogenic microbes. Factors such as season can influence bacterial communities associated with macroalgae: for example, Serebryakova et al. (2018) observed temporal shifts in the bacterial community of *Sargassum muticum*, with large abundances of *Rhodobacteraceae* and *Loktanella* in winter months, but prevalence of *Pirellulales* in summer months.

Despite the growing recognition of the potential role of microbes in the functioning of coral reefs, our understanding of microbial communities of macroalgae is still in its infancy. Previous studies investigating macroalgae microbiomes in tropical environments assess the effect of macroalgae microbial communities on surrounding corals (Smith et al. 2006, Vermeij et al.

2009b, Barott and Rohwer 2012, Barott et al. 2012) rather than solely determining the macroalgae microbiome. Whilst it is important to understand the role of macroalgae microbes in coral-algal interactions, it is also imperative to characterise macroalgae host microbiomes that can act as a comparative baseline for environmental change. Therefore, to understand the ways in which microbes contribute to persistence of macroalgae abundance on coral reefs, it is crucial to know what microbes associate with macroalgae.

The Great Barrier Reef (GBR) (Australia) has been subjected to a number of global and local stressors in recent years that have led to a significant decline in coral cover (De'ath et al. 2012, Hughes et al. 2017, 2018, Matthews et al. 2019, Mellin et al. 2019). Magnetic Island, a high continental island approximately 8km from the Queensland coast in the Central GBR, is surrounded by fringing reefs and has previously been described as having a mixed coral and macroalgae community (Morrissey 1980). *Sargassum* species are common on degraded and inshore reefs of the GBR (Martin-Smith 1993, Schaffelke and Klumpp 1997), and has been recorded as the dominant macroalgae on reefs around Magnetic Island (AIMS Data Centre, 2019).

Sargassum is a genus of brown canopy-forming macroalgae, with numerous species distributed across temperate and tropical marine environments (McCourt 1984). *Sargassum* has a macroscopic thallus, differentiated into a basal holdfast, 'stem'-like primary axis, leaves and air bladders (Mattio et al. 2008). *Sargassum* is covered in microscopic biofilm thought to have a distinct microbial community from the host macroalgae and be important for physiological processes (Dobretsov 2009). On coral reefs, *Sargassum* species grow sub-tidally attaching to a variety of substrate (coral, rock, rubble, shells) using the holdfast mechanism (Loffler et al. 2018). Tropical populations of *Sargassum* species undergo seasonal cycles of growth and senescence (Martin-Smith 1993). Summer growth and abundance of *Sargassum* can blanket

inshore reefs from October-March, with winter senescence starting after the algae has reproduced (Martin-Smith 1992). *Sargassum* rarely completely senesces on reefs where environmental stressors are chronic and persistent, and in regions where this is the case, the reef can be left dominated by *Sargassum* species year round (Ceccarelli et al. 2018).

This study investigates the bacterial communities associated with *Sargassum* biofilm and tissue. In particular we characterised the microbial community of the different *Sargassum* regions (Figure 2.2) using 16S rRNA gene amplicon sequencing and a range of microscopy techniques to visualise microbial structures associated with the *Sargassum* leaf (fluorescent *in situ* hybridisation, scanning electron microscopy and light microscopy).

2.3 Methods

2.3.1 Study site and sample collection

Thalli of *Sargassum polycystum* (n=5) and *Sargassum aquifolium* (n=5) were collected from Geoffrey Bay, Magnetic Island on 31/1/2018 (Figure 2.1, 19º 09’ 09.90” S, 146º 52’ 03.28” E) Each thallus was approximately 1 m in height, and the entire thallus from holdfast to tip was collected from 3-5 m depth using sterile gloves, sterile scissors and sterile chisel. Each thallus collected was a minimum of 10 m away from the nearest live coral to minimise the risk of any coral-algal interactions influencing the samples. Where the holdfast could not be taken, the thallus was collected as close as possible to the base of the alga, which constituted the basal growth (Table 1). Samples were collected under the G15/37574.1 and G16/38348.1 permits issued by the Great Barrier Reef Marine Park Authority.

Table 1 Number of *Sargassum* region replicates for *S. aquifolium* and *S. polycystum.* N represents the number of individual replicates collected with one sample obtained for each region per replicate.

Sargassum aquifolium		***Sargassum polycystum***	
Biofilm	n = 5	Biofilm	n = 5
Leaf	n = 5	Leaf	n = 5
Stem	n = 5	Stem	n = 5
Basal growth	n = 3	Basal growth	n = 5
Holdfast	n = 3	Holdfast	n = 2

The holdfasts still attached to samples were scraped clean of sediment and rock using sterile scalpels before sample preservation. Each thalli collected for DNA extraction was immediately rinsed in sterile seawater to remove loosely attached or seawater microbes from the surface, then preserved in salt saturated EDTA-DMSO (Table S1) in the field and subsequently stored on ice and later kept at -20ºC.

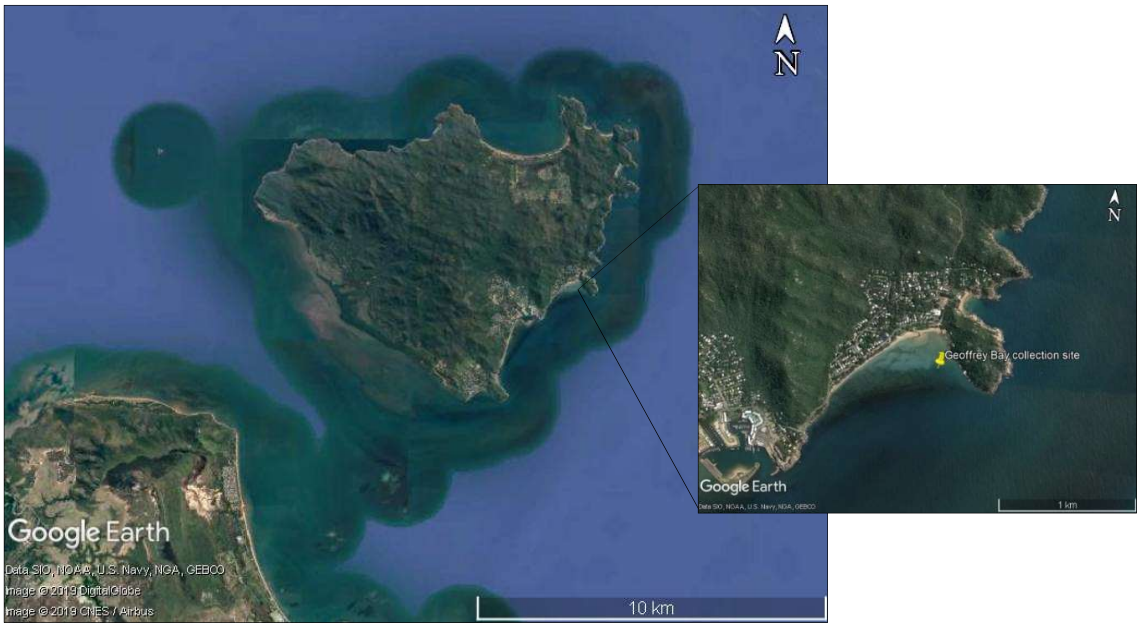


Figure 2.1 Study site location at Magnetic Island, Great Barrier Reef, Australia (Google Earth, 2016, V 7.3.2.5776)

Seawater surrounding the *Sargassum* samples was also collected. Briefly, duplicate samples consisting of 2L of seawater was collected from approximately 3m depth and 2m above the substratum, and filtered through a 0.22 µm Sterivex filter (Merck Millipore). Filters were stored on ice in the field, returned to the laboratory within 1-2 h, and subsequently stored at -20°C until processed further to extract total genomic DNA.

Prior to DNA extraction, each algal sample was dissected into the desired region of *Sargassum*; leaf, stem/axis, biofilm, basal growth and holdfast (Figure 2.2). The leaf, stem, basal growth and holdfast were sampled with the biofilm intact, however the biofilm was also removed from the *Sargassum* thalli and sampled as its own region. To remove biofilm from each sample, a 15-20 cm section of the thallus was placed into 10 mL 1X PBS solution in 15mL polypropylene tubes and sealed with Parafilm. The polypropylene tubes containing samples were vortexed briefly, then placed into a spinning incubator (RATEK Hybridization Oven, speed 10) for 12 h at 37°C (Glasl et al. 2019). Post-incubation, each sample was vortexed briefly, and 2 mL of liquid was aliquoted into sterile 2 mL collection tubes centrifuged for 15 min at 5000 x g. The supernatant was discarded (careful to avoid the pellet), and the sample processing was repeated in the same 2 mL collection tubes until all the liquid from the polypropylene tubes was processed. On the final centrifuge round, the supernatant was removed, and total genomic DNA was extracted from the remaining pellet.

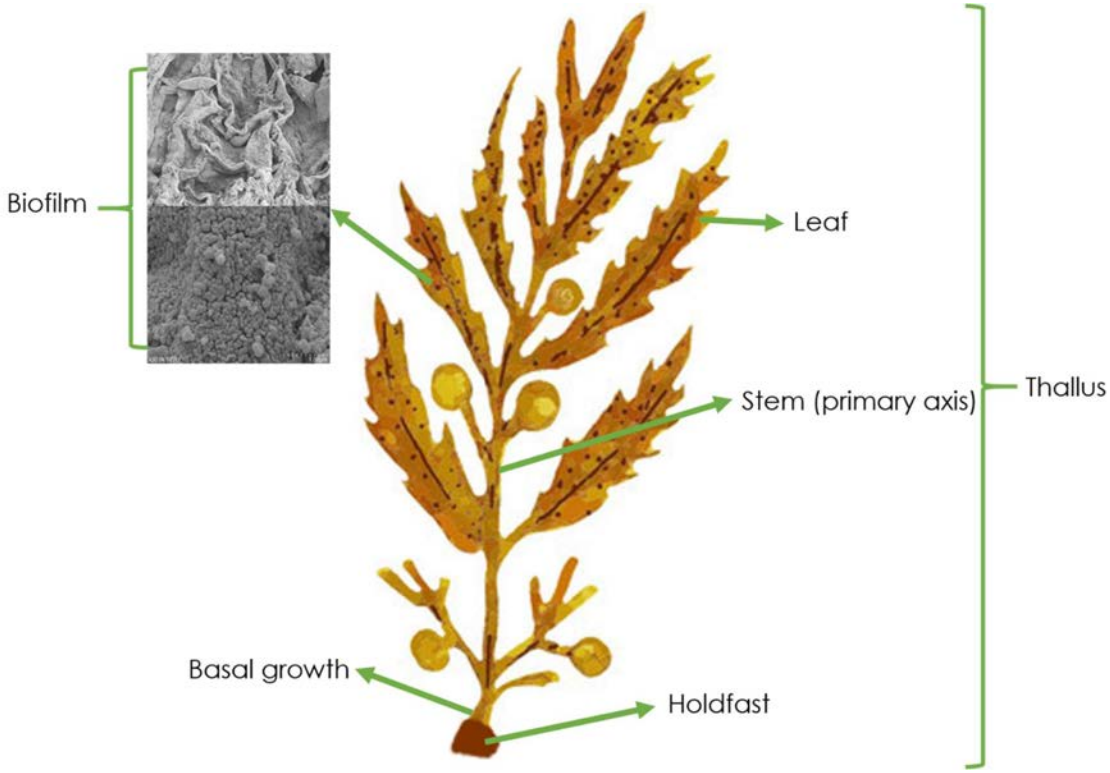


Figure 2.2 Schematic of the *Sargassum* regions sampled in study (credit to G. Al Moajil-Cole).

2.3.2 Fluorescent *in situ* Hybridisation and Scanning Electron Microscopy

Sargassum species samples collected for all microscopy techniques were first rinsed with sterile artificial seawater. *Sargassum* leaves were separated from the algae thallus and then preserved in 4% paraformaldehyde for 10 h. After fixation, samples were stored at 4°C in 50:50 1XPBS:100% ethanol.

Histology was used to visualise internal microbial structures with 3 *Sargassum* leaf samples longitudinally embedded and stained with Alician Blue Safarin (Demarco 2017, Jensen et al. 2018). Samples were visualised using a Leica DM750 microscope, and images obtained with a Leica ICC50W camera using Leica LAZ EZ (V3.4) software.

Scanning electron microscopy (SEM) was used to visualise external structures on the *Sargassum* leaf surface. *Sargassum* leaf samples for SEM (n=2) were dehydrated through an ethanol dehydration series (70% - 100% ethanol), increasing by 5% increments for 10 min at each ethanol concentration. Samples were stored in an open polypropylene tube in silica gel beads to air dry overnight. Dried samples were mounted on aluminium stubs and coated with platinum. The specimens were observed and photographed using a scanning electron microscope (Hitachu SU5000) at 3(kV).

FISH was used to visualise bacterial distribution on the *Sargassum* leaf surface. *Sargassum* leaf samples for FISH were dehydrated through the same ethanol dehydration series as SEM sample preparation. One leaf was used for each visualisation time point and dissected into three sections. Auto-fluorescence trials showed peaks in background fluorescence emissions between 650–680 wavelengths. Therefore, the 488 and 561 lasers were chosen to excite selected probes. For each visualisation time point, 4 samples were prepared: 1 x *Sargassum* leaf section with CY3 EUB 338 probe, 1 x *Sargassum* leaf section CY3 NONEUB 338 probe, 1 x *Sargassum* leaf section with no probe (negative), 1 positive slide (*Escherichia coli* culture).

Hybridisation of probes onto *Sargassum* samples was completed using a protocol amended from Hugenholtz et al. (2001) and Wada et al. (2016). The FISH samples did not need to be de-waxed as the samples used were just the fixed leaves (Zhang et al. 2015). After dehydration samples were left to air dry for 10 min. Samples were placed into a sterile centrifuge tube, and 250 µL of freshly prepared hybridisation buffer heated to 46^{o}C was added (Table S2). 25 µL of oligonucleotide probe (CY3 EUB 338 (I, II, III) and NONEUB 338) was added to the sample at a final concentration of 25 mg mL-1. The samples were incubated at 46^{o}C for 16 h in the dark in a hybridisation oven to allow enough time for probes to penetrate the leaf surface. Post-incubation, samples were immediately rinsed with freshly prepared wash buffer (Table S3)

heated to 48ºC and held in a centrifuge tube with 2 mL of wash buffer at 48ºC for 10 min. The sample was soaked in cold filtered water for 10 s, and then thoroughly dried using compressed air. Samples were placed onto sterilised histology slides using Citifluor Antifadent Mounting Solution and sealed with a cover slip. The samples were stored in the dark and visualised on a confocal microscope (Zeiss LSM 710, ZEN software) within 4 h. The positive bacterial culture samples were smeared and air-dried onto Superfrost Plus adhesive slides. A hydrophobic pen was used to create a barrier around the cells, and 90 µL of freshly prepared hybridisation buffer pre-heated to 46ºC was applied to the culture. 10 µL of oligonucleotide probe was added at a final concentration of 25 ng mL-1. The positive slides were placed in an opaque box with paper towel soaked with the remaining hybridisation buffer. The positive slides were incubated in the dark at 46ºC for 16 h. Post incubation, the slides were immediately rinsed with wash buffer preheated to 48ºC then placed into a 50 mL polypropylene tube with preheated wash buffer and held at 48ºC for 10 min. The positive slides were then rinsed with cold filtered Milli-Q water to remove excess salts. The slides were thoroughly dried using compressed air and mounted with Citifluor Antifadent Mounting Solution, and sealed with a cover slip. The leaf and positive samples were stored in the dark and visualised using a confocal microscope within 4 h.

2.3.3 Total genomic DNA extraction

DNA was extracted from each region (leaf, stem, biofilm, holdfast, basal growth) using the *Qiagen DNeasy PowerBiofilm Kit* following the manufacturer's instructions, with the exception of the following minor alterations: 2 mL of 'NEXT ADVANCE' zirconium oxide 0.5mm beads were added to each Power Biofilm bead tube prior to the sample being added to the tubes; 20-40 mg of tissue was used. The leaf, stem, basal growth and holdfast were dissected into the smallest pieces possible using a scalpel until it reached a 'sludge-like' consistency, and added directly into the Power Biofilm bead tubes followed by Solution MBL; the whole biofilm

pellet was re-suspended in Solution MBL. All of the re-suspended biofilm was transferred directly into the Power Biofilm bead tubes; after adding Solution FB, each sample was incubated at 65ºC for ten minutes; all samples underwent bead-beating for five minutes; when required to add Solution IRS, 200 µL was added to every sample and then incubated at 4ºC for ten minutes; the final elution was completed in two 50 µL elutions, instead of one 100 µL elution to ensure as much DNA as possible was removed from the spin column filter. All other steps were kept consistent with the DNeasy Power Biofilm Kit protocol.

DNA was extracted from the Sterivex filters for seawater microbial analysis. The filters were removed from their outer housing using sterile pliers. Once removed, a quarter of the filter paper was cut from the inner housing using a sterile scalpel blade and placed into the PowerBiofilm bead tubes using sterile tweezers. The DNA extraction was completed using the *DNeasy PowerBiofilm Kit* and using the same modifications as the tissue/biofilm extractions.

2.3.4 PCR and 16S rRNA gene amplicon sequencing

The 16S rRNA gene region was amplified using 799F (5'– AACMGGATTAGATACCCKG – 3') and 1193R (5'– ACGTCATCCCCACCTTCC –3') primers (fragment ~400bp), which target the V5, V6 and V7 regions of the 16S rRNA gene and avoid chloroplast cross amplification (Bodenhausen et al. 2013, Vieira et al. 2016a, Greff et al. 2017). Each primer was fused with the forward and reverse Illumina overhang sequences (forward overhang: 5'– TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG–3', reverse overhang: 5'– GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG–3') (Illumina 2013). The following amplification protocol was used: after an initial denaturation at 95ºC for 1min, conditions were 35 cycles of denaturation at 95ºC for 15s, annealing at 55ºC for 15s and extension at 72ºC for 15s. The final extension was at 72ºC for 15min. The 50 µL reaction contained 1 µL of each primer (20µM), 25µl MyFi 2X Mix (Bioline), 21 µL Milli-Q water and

2 µL template DNA (1:10 dilution). PCR product was submitted to Ramaciotti Centre for Genomics, UNSW Sydney for bead purification, library preparation (using Illumina MiSeq DNA library preparation protocol), and paired-end (2x250) Illumina MiSeq sequencing.

2.3.5 16S rRNA analysis and bacterial community diversity

Sequence reads of all samples were processed using QIIME2 (Caporaso et al. 2010). Sequence reads returned from sequencing were demultiplexed and rarefied. Reads were filtered for quality and chimeric sequences using DADA2 (Callahan et al. 2016). Taxonomic classification was assigned using a naïve Bayes classifier, trained on the extracted regions of interest from the SILVA 16S rRNA (99) reference alignment (132 QIIME release (Quast et al. 2013)). All sequences classified as chloroplast, mitochondria or Eukaryota were removed. The resulting amplicon sequence variant (ASV) table was used for statistical analysis in Calypso (Zakrzewski et al. 2016) and in R Studio (R Core Team 2017).

Alpha diversity was calculated using Shannon's diversity index and Faith's richness index. Patterns in microbial community composition among species and *Sargassum* region were visualized using non-parametric multidimensional scaling (NMDS). Permutational multivariate analysis of variance (PERMANOVA) and PERMADISP were used to identify differences in the microbial community composition between the two *Sargassum* species and among the five regions of *Sargassum* sampled. The percent relative abundance of each microbial phylum, class and family present in all samples was calculated. The most abundant phyla across both *Sargassum* species, region and seawater were plotted, with 'other' accounting for <1% of remaining samples. The most abundant bacterial families across all samples and replicates in each region of each algal species were plotted against their bacterial class. ASV analysis was completed by calculating the most abundant taxa associated with 100% of samples for each *Sargassum* species and region, identified to the lowest possible

taxonomic level before 'unknown' or 'unclassified'. Compiling the most abundant taxa for each region and species resulted in a final list of 23 important ASVs. ASVs were BLASTed against the NCBI database to ascertain habitats the ASVs had been previously associated with.

2.4 Results

2.4.1 Visualising microbial communities on *Sargassum* leaf sections

No internal microbial cell structures were observed using histology (see Figure S1) and therefore, microscopy approaches focused on microbial structures visualised on the leaf surface. Scanning electron microscopy (SEM) photomicrographs revealed the presence of a rich microbiota community residing on the *Sargassum* species leaf surface biofilm. Bacterial cell morphologies were observed, with abundant bacterial coccoid-like clusters across the leaf surface (Figure 2.3a). Furthermore, structures similar to prosthecate filaments were associated with the biofilm community (Figure 2.3b). A range of microscopic structures consistent with diatoms were commonly observed on the leaf surface (Figure 2.3c, d). Through SEM, a number of ostiole structures were visualised (Figure 2.3e); microscopic openings on the leaf surface associated with *Sargassum* reproduction. Lastly, possible fungal cell morphologies were also observed (Figure 2.3f), supporting the probable diversity of microbiota associated with tropical variants of *Sargassum* species.

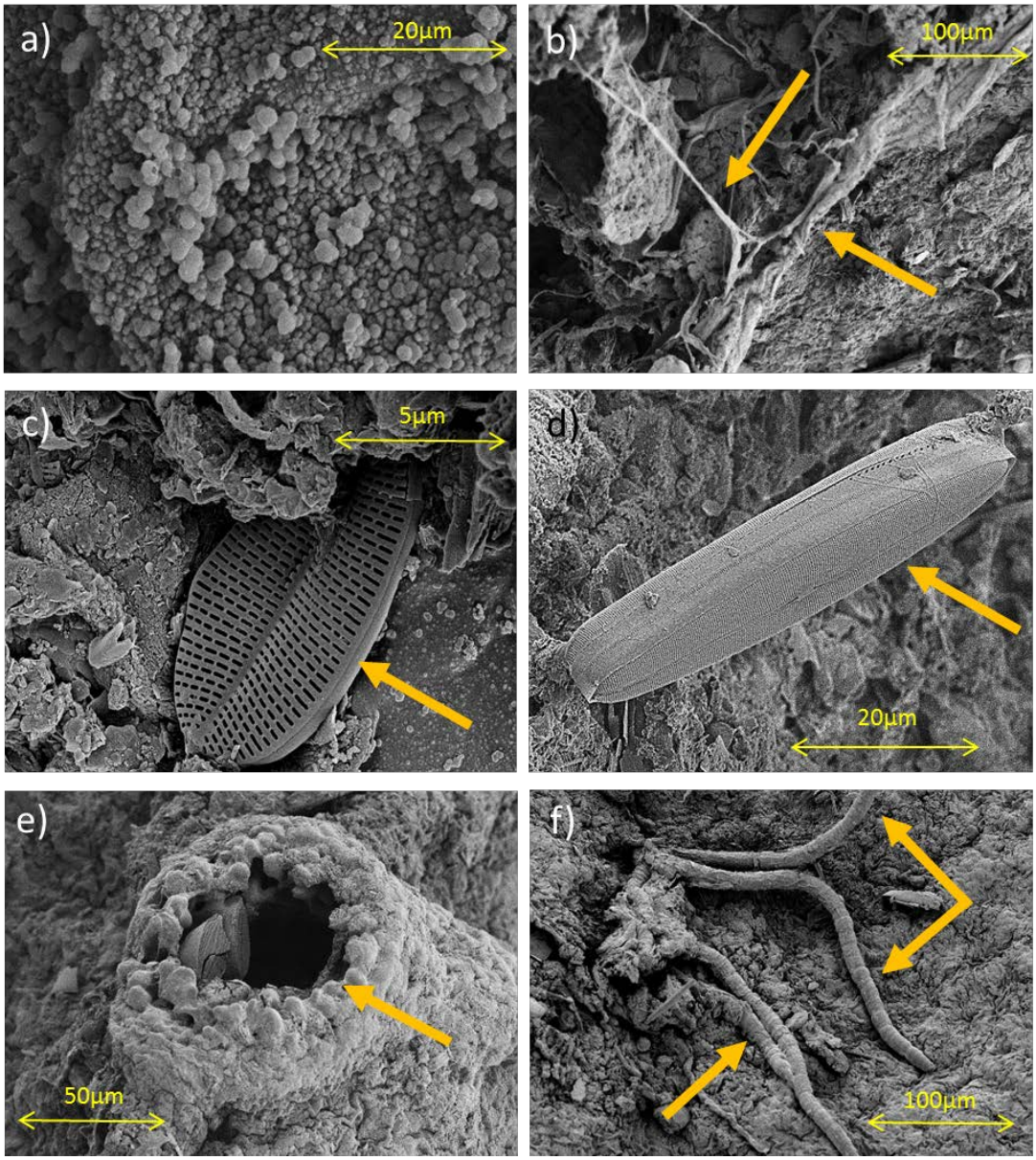


Figure 2.3 SEM photomicrographs: a) Coccoid-like bacteria clusters (magnification: 22,000X). b) Possible prosthecate filaments associated with bacteria biofilm formation (magnification: 250X). c) Pennate diatom structure (magnification: 8000X). d) Elongate diatom structure (magnification: 2200X). e) Ostiole structure associated with *Sargassum* species reproduction (magnification: 700X). f) Possible filamentous fungal structures (magnification: 350X).

Confocal micrographs of fluorescent signals revealed the presence of high autofluorescence on the *Sargassum* sp. leaf surface. Autofluorescent signals (Figure 2.4a, b) associated with the *Sargassum* species leaf surface were strongest using the confocal microscope 480 laser. The

confocal microscope 561 and 630 lasers were therefore used to excite the CY3 EUB338 (I, II, III) and CY3 NON-EUB338 probes. A variety of epiphytic bacterial structures were observed in relatively high abundance across the *Sargassum* species leaf surface (Figure 2.4c). Varying bacterial morphologies were also detected, including filamentous cells (Figure 2.4d).

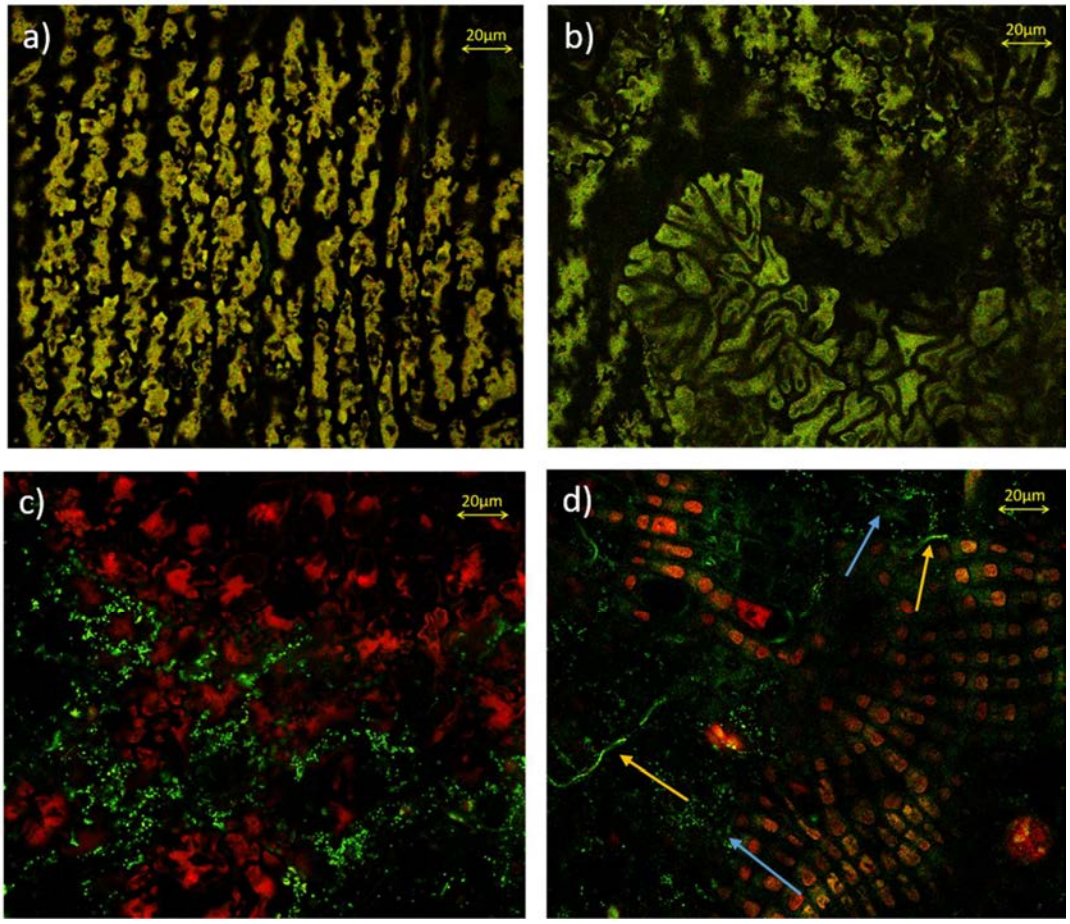


Figure 2.4 Confocal micrographs: a) Autofluorescence of *Sargassum* leaf cells with no probe attached (magnification: 60X). b) Autofluorescence of *Sargassum* leaf cells detected as negative control with NON-EUB338 probe attached (magnification: 60X). c) Red is used to visualise autofluorescence of *Sargassum* leaf cells, green is used to visualise positive bacteria signal of coccoid-like clusters on the *Sargassum* leaf surface (magnification 40X). d) Red is used to visualise autofluorescence of *Sargassum* leaf cells, green is used to visualise positive bacteria signal of probably filamentous structures (yellow arrow) and coccoid-like clusters (blue arrow) (magnification: 60X).

2.4.2 Bacteria community diversity comparisons

A total of 3,531,584 16S rRNA gene sequence reads were recovered from all 48 samples processed. Following filtering and rarefaction, 790,728 high quality reads were subsequently used for taxonomic classification (Table 2). Rarefaction analysis confirmed sampling depth was sufficient to estimate the total diversity of each sample (Figure 2.5).

Table 2 List of species, samples and sequence reads, highlighting the quality reads used for taxonomic classification

Species	Sample	No. raw reads	No. cleaned reads	% Removed
S. aquifolium	Basal growth, #1	214664	171314	20.19
S. aquifolium	Biofilm, #1	241288	189384	21.51
S. aquifolium	Holdfast, #1	286021	226102	20.95
S. aquifolium	Leaf, #1	252037	194467	22.84
S. aquifolium	Stem, #1	245641	193917	21.06
S. aquifolium	Basal growth, #2	218063	167477	23.2
S. aquifolium	Biofilm, #2	219656	180022	18.04
S. aquifolium	Holdfast, #2	192510	151488	21.31
S. aquifolium	Leaf, #2	400779	323126	19.38
S. aquifolium	Stem, #2	348032	274590	21.1
S. aquifolium	Basal growth, #3	249445	187347	24.89
S. aquifolium	Biofilm, #3	343339	274079	20.17
S. aquifolium	Holdfast, #3	165535	106608	35.6
S. aquifolium	Leaf, #3	533420	454916	14.72
S. aquifolium	Stem, #3	345171	275708	20.12
S. aquifolium	Biofilm, #4	183980	145460	20.94
S. aquifolium	Leaf, #4	249168	204252	18.03
S. aquifolium	Stem, #4	287184	228052	20.59
S. aquifolium	Biofilm, #5	202173	127111	37.13
S. aquifolium	Leaf, #5	176053	143410	18.54

S. aquifolium	Stem, #5	339948	275744	18.89
S. polycystum	Basal growth, #1	470169	379029	19.38
S. polycystum	Biofilm, #1	346248	276040	20.28
S. polycystum	Leaf, #1	241975	193613	19.99
S. polycystum	Stem, #1	227108	182216	19.77
S. polycystum	Basal growth, #2	561943	445213	20.77
S. polycystum	Biofilm, #2	351156	283352	19.31
S. polycystum	Holdfast, #2	337185	268438	20.39
S. polycystum	Leaf, #2	359557	285382	20.63
S. polycystum	Stem, #2	229068	176105	23.12
S. polycystum	Basal growth, #3	344181	266619	22.54
S. polycystum	Biofilm, #3	372023	309074	16.92
S. polycystum	Leaf, #3	306790	249740	18.6
S. polycystum	Stem, #3	225725	173834	22.99
S. polycystum	Basal growth, #4	210115	148743	29.21
S. polycystum	Biofilm, #4	265115	212587	19.81
S. polycystum	Leaf, #4	281112	231389	17.69
S. polycystum	Stem, #4	226125	183021	19.06
S. polycystum	Basal growth, #5	293277	224028	23.61
S. polycystum	Biofilm, #5	260140	211936	18.53
S. polycystum	Leaf, #5	320797	263109	17.98
S. polycystum	Stem, #5	252900	197433	21.93
n/a	Seawater, #1	204988	177276	13.52
n/a	Seawater, #2	231761	188792	18.54

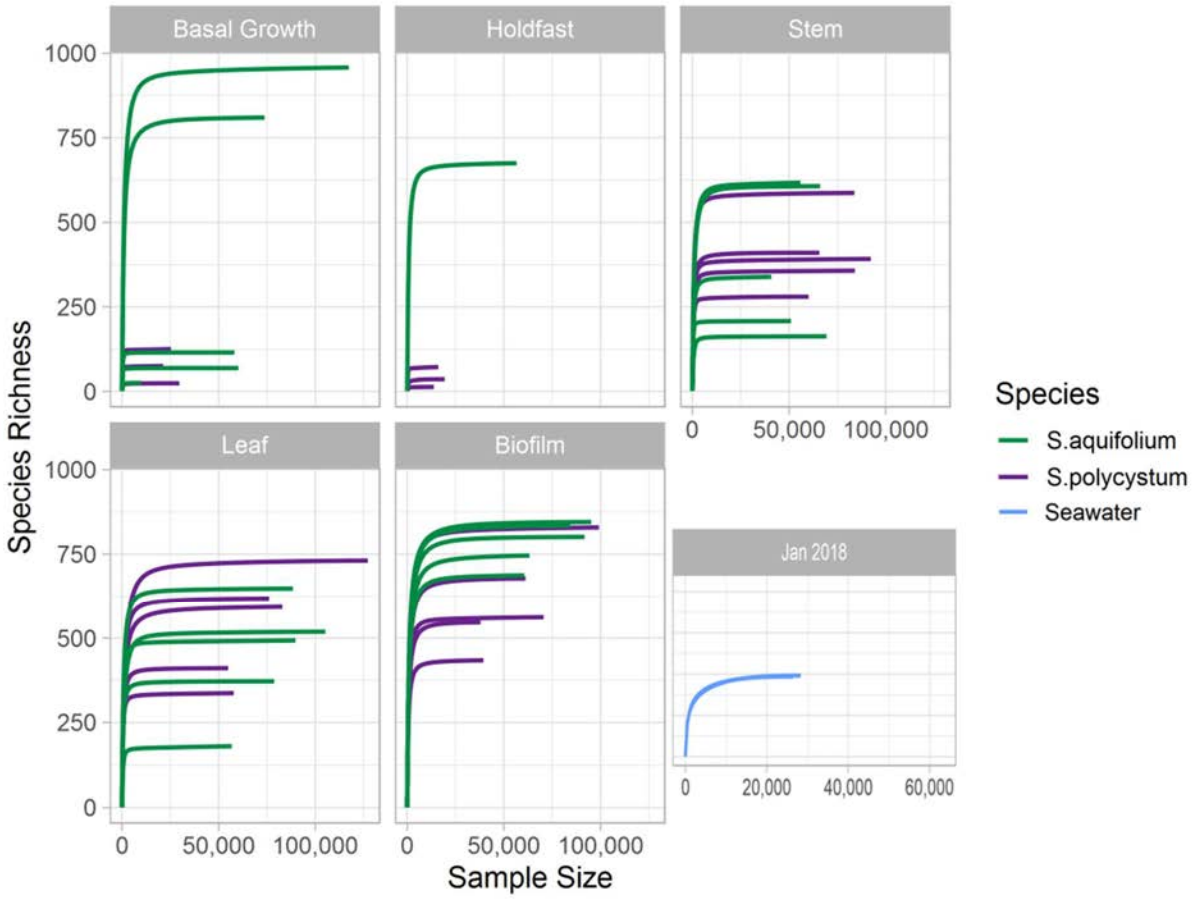


Figure 2.5 Rarefaction curves visualising sampling depth of individual *S. aquifolium*, *S. polycystum* and seawater samples

Shannon's diversity index and Faith's richness index of microbial communities associated with *S. aquifolium* (n=5) and *S. polycystum* (n=5) showed no significant differences (Shannon's – ANOVA: p = >0.05, F = 0.8; Faith's – ANOVA: p = >0.05, F = 1.3) (Figure 2.6a, b). As such, sequences of the regions from each algal species were pooled. Both Shannon's index and Faith's index showed a significant difference in the microbial communities of the separate *Sargassum* regions (Shannon's – ANOVA: p = <0.05, F = 7.3; Faith's – ANOVA: p = <0.05, F = 7.3) (Figure 2.6c, d). Faith's richness index showed the taxa richness of each region: holdfast = 123.47 ASVs per sample, basal growth = 183.39 ASVs per sample, stem = 294.739 ASVs per sample, leaf = 365.47 ASVs per sample, biofilm = 436.14 ASVs per sample. Further

investigation using Kruskal-Wallis pairwise tests, however, revealed significantly higher richness and diversity within only the biofilm compared to the remaining regions, most likely due to outliers within leaf and stem sample groups (biofilm/holdfast, p = <0.05; biofilm/leaf, p = <0.05; biofilm/stem, p = <0.05; biofilm/basal growth, p = <0.05).

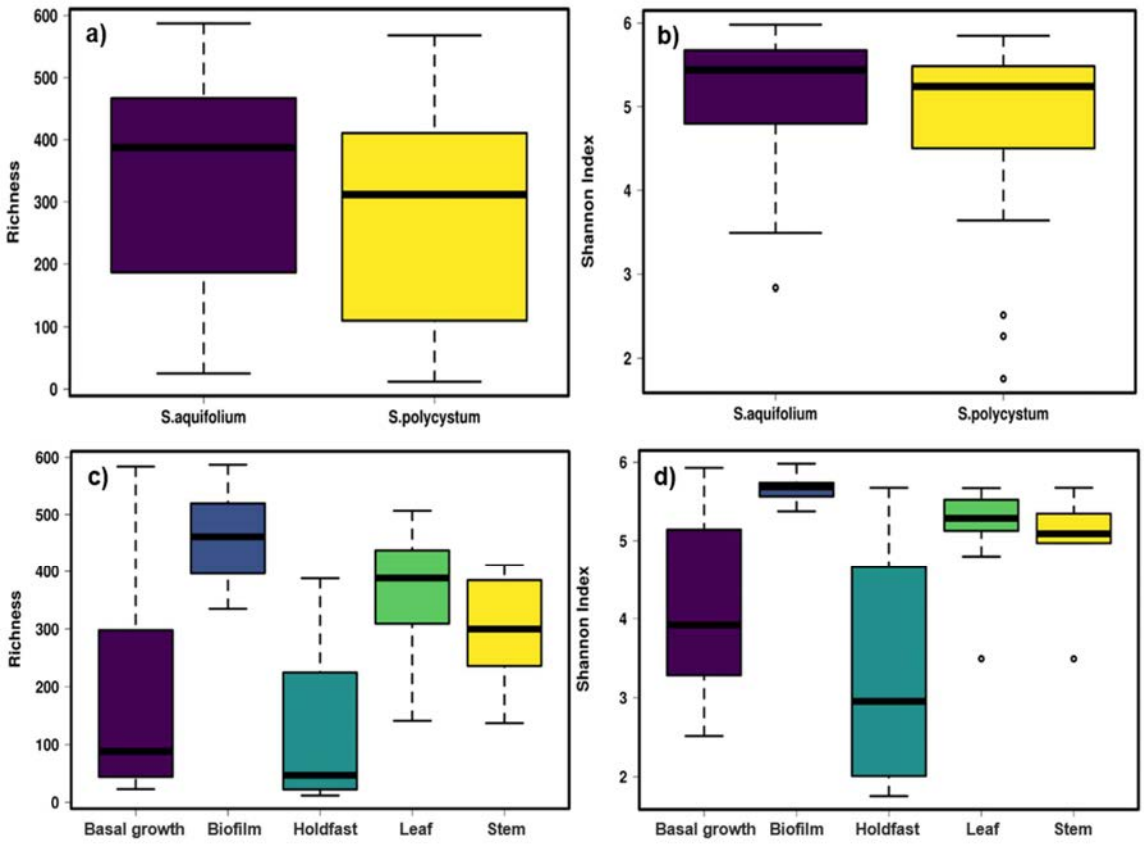


Figure 2.6 Alpha diversity assessment of *Sargassum* species and associated regions. Top panels (a, b) - alpha diversity assessment of *S. aquifolium* and *S. polycystum*. Bottom panels (c and d) - alpha diversity comparison of *Sargassum* regions (basal growth, biofilm, holdfast, leaf and stem) with *Sargassum* species data pooled.

There was no significant difference between bacterial community compositions of the two species (PERMANOVA, $R^2 = 0.036$, p = > 0.05), or with regards to the amount of variability (PERMDISP2, p = 0.285) (Figure 2.7a). Across all samples, bacterial community compositions of the *Sargassum* regions were significantly different (PERMANOVA, $R^2 = 0.285$, p = <0.05), as was the variability between regions (PERMDISP2, p = <0.001) (Figure 2.7b). The samples

from the leaf, stem and biofilm were tightly clustered, suggesting greater bacterial consistency in these regions. Furthermore, the biofilm and leaf samples were clustered, suggesting a high correlation between the leaf and biofilm microbial communities. The samples from the basal growth and holdfast were not tightly clustered. Whilst this could be a result of lower sample size for holdfast and basal growth, it could also suggest a higher degree of variability in microbial community composition of these regions.

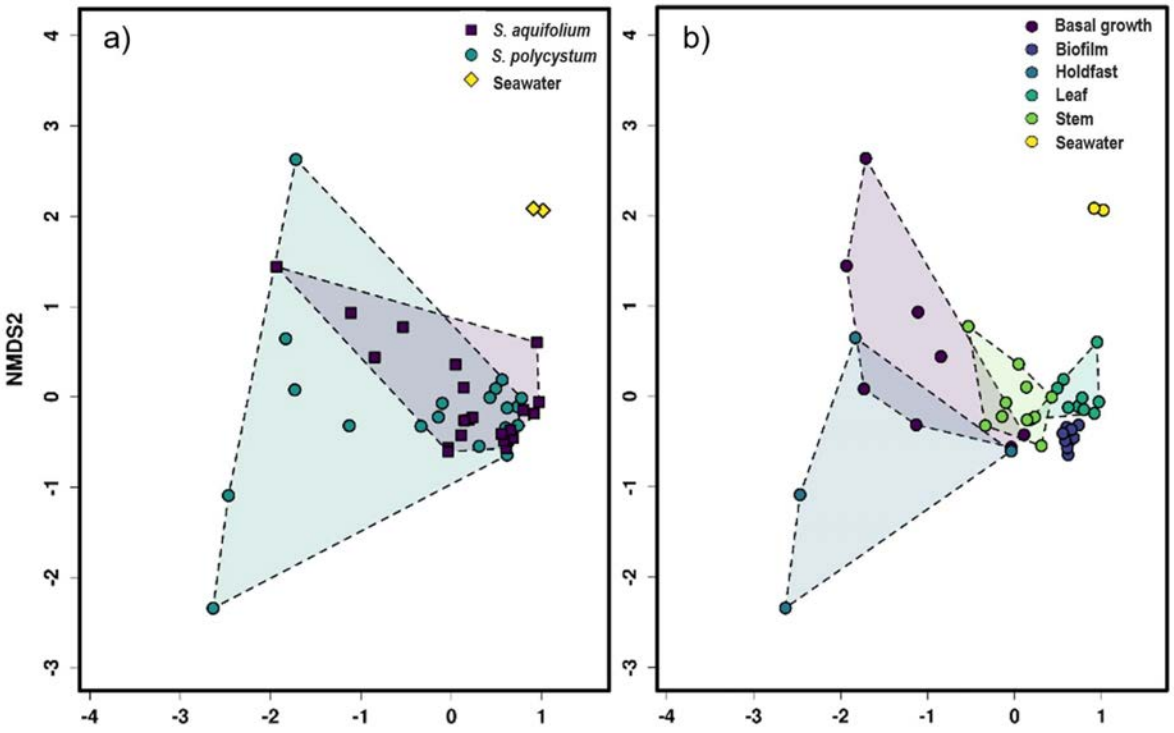


Figure 2.7 Beta diversity assessment of *Sargassum* species and associated regions. a) Community composition comparisons between *S. aquifolium*, *S. polycystum* and seawater. b) Community composition comparisons between regions *(Sargassum* species data pooled) and seawater).

2.4.3 Bacteria community composition

Sequences associated with 21 microbial Phyla were identified within the 16S rRNA gene dataset across both algal species. *Bacteroidetes* and *Proteobacteria* were the most abundant sequences retrieved from samples derived from both species and across all *Sargassum* regions representing between 34-51% and 28-52% respectively. *Bacteroidetes* affiliated sequences were most abundant in the biofilm and least abundant in the holdfast, while *Proteobacteria*

was most commonly found in the holdfast and least commonly found in the stem. Whilst dominant phyla were consistent between *Sargassum* species, *S. polycystum* displayed an overall slightly lower abundance of *Proteobacteria* (34%) compared to *S. aquifolium* (41%), with higher relative abundances of both *Actinobacteria* (*S. polycystum*: 16%, *S. aquifolium*: 12%), *Firmicutes* (*S. polycystum*: 2%, *S. aquifolium*: 0.8%) and *Patescibacteria* (*S. polycystum*: 0.7%, *S. aquifolium*: 0.3%) (Figure 2.8).

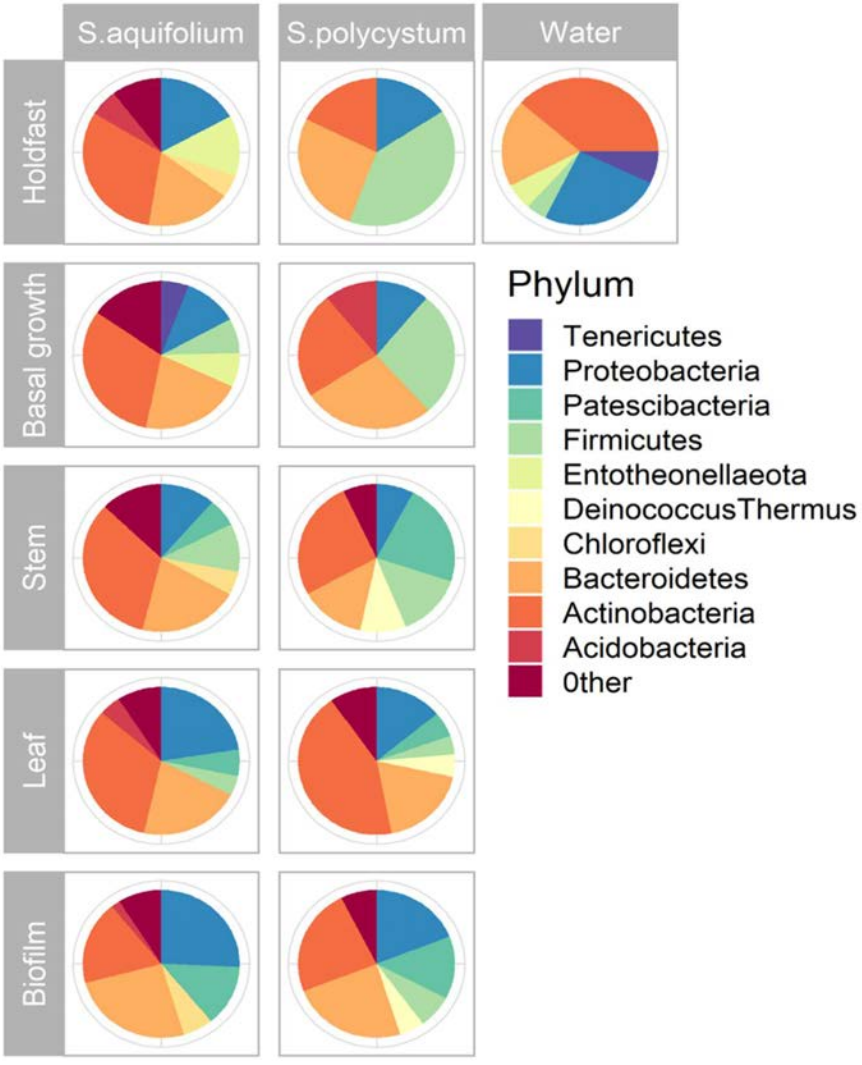


Figure 2.8 The most abundant microbial phyla associated with *S. aquifolium* and *S. polycystum* across regions. "Other" includes phyla representing <1% of all sequences. Relative abundances are the percent values of the total number of sequences.

Within the *Proteobacteria,* sequences associated with *Alphaproteobacteria* and *Gammaproteobacteria* were the most abundant, representing 23% and 15% of retrieved sequences for *S. aquifolium*, and 19% and 13% of all sequences for *S. polycystum* respectively (Figure 2.9). Across both algal species and all *Sargassum* regions, *Alphaproteobacteria* were dominated by sequences associated with the family *Rhodobacteraceae* (*S. aquifolium*: 22%, *S. polycystum*: 16%), while the *Gammaproteobacteria* were not dominated by a single family, but were instead more evenly spread across several families (e.g. *Porticoccaceae*: *S. aquifolium* 1.4%, *S. polycystum* 2.2%; *Alteromonadaceae*: *S. aquifolium* 2.9%, *S. polycystum* 2.1%; *Woeseiaceae*: *S. aquifolium* 5.5%, *S. polycystum* 2.5%; *Vibrionaceae*: *S. aquifolium* 1%, *S. polycystum* 0.5%) (Figure 2.9). While *Deltaproteobacteria* affiliated sequence were present across both algal species, their abundances were low and most commonly found in the holdfast of *S. aquifolium* (4%) and basal growth of *S. polycystum* (3.5%).

Within the class *Bacteroidia* sequences affiliated with several families were found in high relative abundances across both algal species and all regions. For example, *Flavobacteriaceae* represented between 7-32% of all retrieved sequences. Interestingly, while *Flavobacteriaceae* was found across all regions, the highest and lowest relative abundance of sequences was retrieved from the basal growth section of each algal species (*S. aquifolium*: 32%, *S. polycystum*: 7%) (Figure 2.9). *Saprospiraceae* affiliated sequences represented between 2-24% of sequences, and while also present across all *Sargassum* regions, was in higher relative abundances within the leaf and biofilm of both algal species (between 12-24% of retrieved sequences) compared to only 2-7% of the sequences retrieved from the holdfast and basal growth sections of these algal species. Finally, *Microtrichaceae* (class *Acidimicrobiia*) was also found across both algal species and all regions, representing between 2-18% of sequences (Figure 2.9).

Some sequences were only retrieved from specific regions of the macroalgae. For example, members of the Firmicutes phylum including *Clostridiaceae* were observed associated with basal growth of *S. polycystum* (6%), *Lactobacillaceae* from the holdfast of *S. polycystum* (5%) and *Streptococcaceae* from both the basal growth (3.3%) and stem (2.5%) of *S. aquifolium*. The higher relative abundance of taxa associated with *Firmicutes* in the lower macroalgae regions (holdfast, basal growth and stem) of *S. polycystum* and *S. aquifolium*, suggest they may be useful region indicator taxa (Figure 2.9).

BLASTing the most abundant ASVs revealed associations with tropical marine environments, localised from seawater, sediment and coral reef taxa (Figure 2.10). 9 ASVs were associated with both *Sargassum* species and all regions, and included *Gammaproteobacteria*, *Rhodobacteraceae*, *Loktanella*, *Flavobacteriaceae*, *Maritimimonas*, *Saprospiraceae*, *Sva0996 Marine Group* and *Microtrichaceae*. Of these 9 ASVs, 7 were not retrieved from the surrounding seawater samples, suggesting some distinction between the seawater and *Sargassum* bacterial communities, and further suggesting that some bacterial groups are enriched or specific to the *Sargassum* microbiome. The *Gammaproteobacteria* ASV had the highest mean relative abundance (*S. aquifolium*: 19%, *S. polycystum*: 10%) followed by *Flavobacteriaceae* ASV (*S. aquifolium*: 7.6%, *S. polycystum*: 5.9%). Interestingly, whilst *Saprospiraceae* (*S. aquifolium*: 1.8%, *S. polycystum*: 2.8%) and *Loktanella* (*S. aquifolium*: 3.7%, *S. polycystum*: 2.6%) were observed across both *Sargassum* species and all regions, they were found in highest relative abundance within the upper *Sargassum* regions (*Saprospiraceae*: *S. aquifolium* leaf 3.3%, biofilm 2.8%; *S. polycystum* leaf 6.9%, biofilm 3.5%. *Loktanella*: *S. aquifolium* leaf 8%, biofilm, 7.3%; *S. polycystum* leaf 6%, biofilm 4.1%). Differentiation of the upper regions was also observed with the ASVs *Weeksellaceae* and *Bradymonadales*. *Bradymonadales* was observed in the biofilm (*S. aquifolium*: 1.9%, *S. polycystum*: 3.4%), leaf (*S. aquifolium*: 2.2%, *S. polycystum*: 6.6%) and stem (*S. aquifolium*: 3.4%, *S. polycystum*:

2.3%) of both *Sargassum* species. *Weeksellaceae* was present within the leaf (*S. aquifolium*: 2.2%, *S. polycystum*: 4.6%) and biofilm (*S. aquifolium*: 1.8%, *S. polycystum*: 4.8%) of both species, but only present in the stem of *S. aquifolium* (1.1%). Differences in microbial patterns of the lower regions were also observed. For example, the ASV *Geobacter* was only found in the basal growth (2%) and holdfast (1.2%) of *S. aquifolium*. Moreover, whilst *Gammaproteobacteria* was observed across all regions, it was found in highest relative abundance in the holdfast (8.8%) and basal growth (6.1%).

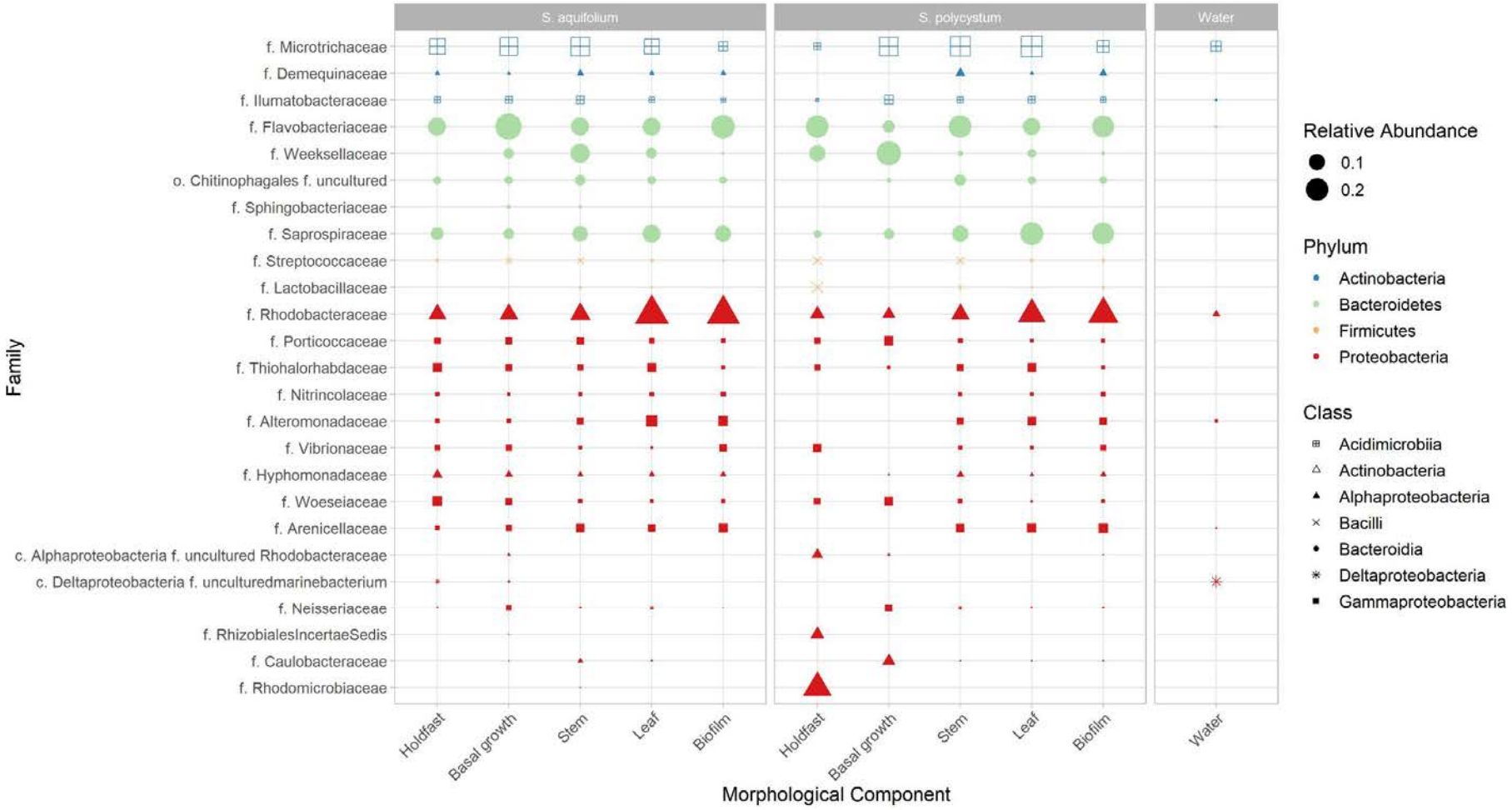
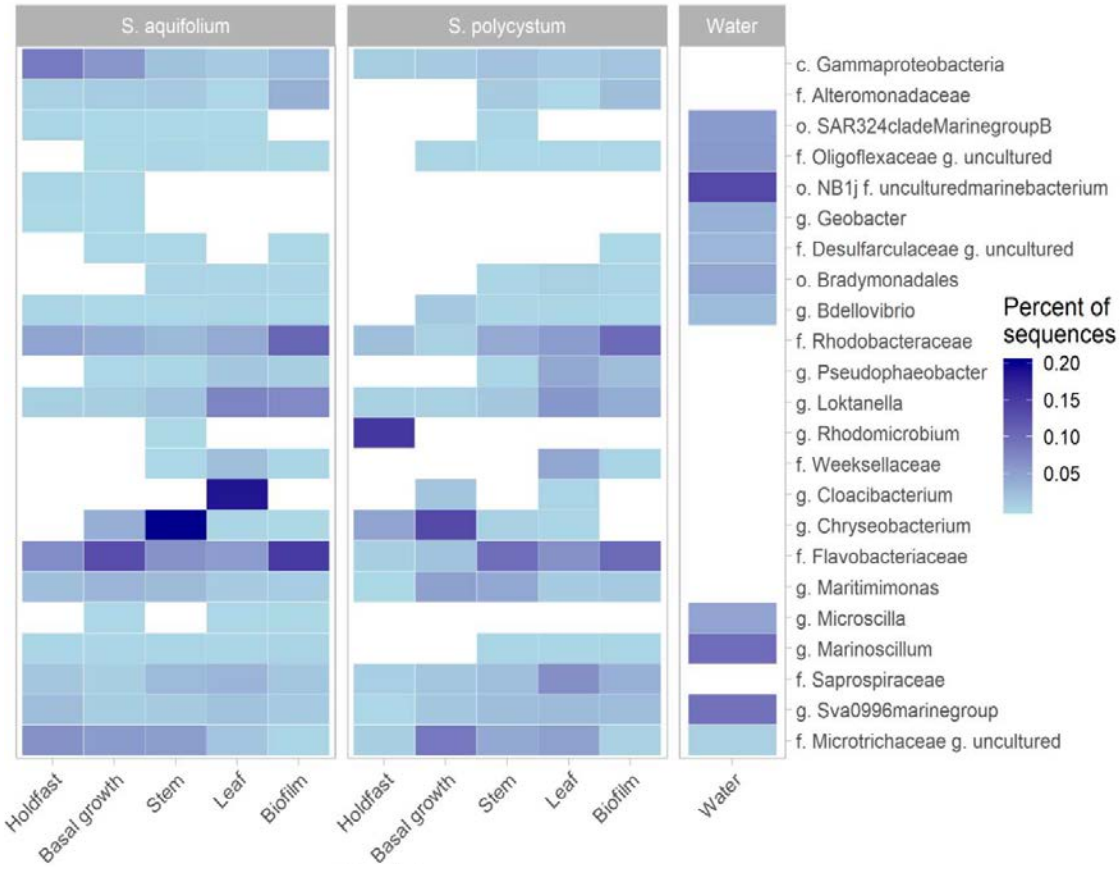


Figure 2.9 Top 25 most abundant bacterial families associated with all samples and replicates of both *Sargassum* species and regions. Families grouped by associated phylum (colour) and differentiated by class (shape). Percentages are the relative abundances of total sequences.



ASV	Source	BLAST ID/Locus	Reference
c. Gammaproteobacteria 499649771ca0b7d63fa5ec62efefbc2f	Macroalgae	GU451359	Lachnit et al., 2011
f. Alteromonadaceae c0c1f586fc8db6c8cfc10d6e42377f77	Seawater	MG002289	Kumar et al. 2017
o. SAR324 Marine Group B 0ecc9c38248df19f2ad91d0ec9513ee4	Biofilm	JN427648	Harris et al. 2013
f. Oligoflexaceae f2e02bd865762e1ad8271d75c97edba5	Seawater	JN868830	Peng and Xiao, 2011
o. NB1j d4d0e0bd5977f7d2bc8e63cd8986343d	Sediment	DQ256663	Uthicke, S., 2005
g. Geobacter c7c321890d1df64335a7f7d530cc4c29	Macroalgae	KU633099	Aires et al., 2016
f. Desulfarculaceae 8a7d2ca4f42a0ef85f1616c600862e89	Sediment	EU491304	Santelli et al., 2008
o. Bradymonadales 716b9ce52780d627c22fde7650f0a56a	Macroalgae	KU632591	Aires et al., 2016
g. Bdellovibrio 37fb1f5be14d8b0fccf21129ad070e19	Macroalgae	AF259630	Meusnier, I. P., 2000
f. Rhodobacteraceae 2119714dad0a7fce605ce13dffb5a34a	Coral	GQ301259	Littman et al., 2009
g. Loktanella ddbf621e80c0a581b60503ac75086fb2	Seawater	JF769741	Chu and Zhang., 2011
g. Rhodomicrobium 655c2e7292b93b919783e01a09d183b7	Sediment	KJ015812	Ding and Nie., 2013
f. Weeksellaceae 637021818b1df4c4a1fc51750dad4f6f	Seawater	DQ256356	Gihring et al., 2005
g. Cloacibacterium 745bae15cd94c0917b4f4761afcab3c3	Sediment	FJ497662	Sudek et al., 2008
f. Flavobacteriaceae 9da7adbd43396a785d491ecbd0b30e29	Coral	GU118405	Sunagawa et al., 2010
g. Maritimimonas 982e32abf71f126ea532a1e08cea5a68	Coral	MK176283	Goldsmith et al., 2019
g. Microscilla 0d0664b467ca63d18052a438cc4a064e	Coral	JN791011	Johnson et al., 2008
f. Saprospiraceae 7d270dbfdeaad85ce26964cbe8e2ebe7	Macroalgae	KU620303	Aires et al. 2016
g. Sva0996 Marine Group fb3f8774daef30f40b5987839aa8492a	Macroalgae	HM474951	Wang et al., 2010
f. Microtrichaceae 8367c2cf7f1318cb97a897378d2c2fc8	Sponge	AY948355	Wichels et al., 2006

Figure 2.10 ASV analysis of the top 23 more abundant taxon (not including taxon labelled 'other' or 'unclassified') in 100% of samples present across *S. aquifolium, S. polycystum*, seawater and each *Sargassum* region. The darker the blue, the most abundant the ASV and where white, the ASV was not present. Corresponding table: BLAST information of each taxa extracted from the NCBI database. Percentages are the relative abundances of total sequences.

2.5 Discussion

2.5.1 Similarity between the *S. aquifolium* and *S. polycystum* bacterial communities

Identification of the algal microbiome can help identify their role in promoting host health, similar to the coral holobiont (Rohwer et al. 2002, Thurber et al. 2009, Glasl et al. 2016). This holobiont concept has been successfully applied to benthic macroalgae (Barott et al. 2012, Egan et al. 2013), and algal-associated bacterial communities can be specific to the type of macroalgae investigated (Singh and Reddy 2016, van der Loos et al. 2019). Here we found the bacterial communities of the two *Sargassum* species sampled were highly diverse, but the overall diversity and bacteria community composition of both algal species were similar. Previous research has indicated the bacterial communities of different algal functional groups vary, though the bacterial communities of algae within each functional group remained similar (Barott et al. 2011). Thus, the similarity of the bacteria community between *S. aquifolium* and *S. polycystum* of the same genus and algal functional group is consistent with these previous studies.

The epiphytic bacterial community profiles of different macroalgae species across temporal and spatial scales has been previously profiled (Tujula et al. 2010, Lachnit et al. 2011, Campbell et al. 2015, Mancuso et al. 2016), suggesting the macroalgae sampled from the same site and time host similar bacteria communities. Both *Sargassum* species were collected at the same time point and from the same location, which may explain the consistency in the bacterial communities between *S. aquifolium* and *S. polycystum*. Campbell et al. (2015) suggested a 'lottery model' whereby host bacterial communities can be influenced by surrounding environmental conditions (such as temperature) to enable host success in the given environment. Furthermore, on coral reefs, Glasl et al. (2019) revealed host-associated

microbiomes can be affected by different environmental parameters, suggesting community assembly patterns can be variable between conspecific individuals from different environmental conditions. Further research investigating the *Sargassum* microbiome should include samples collected from different locations and time points to assess to what extent the surrounding environment may influence the host bacterial communities.

Results from this study showed *Sargassum* species were host to a diverse bacterial community distinct from seawater. Recent research has highlighted the *Sargassum* species biofilm bacterial community has a more varied response to environmental changes compared to seawater, and remains distinct from the seawater microbiome throughout fluctuations in the surrounding environment (Glasl et al. 2019). Such variability within host microbiomes can be attributed to increased niche space providing habitat for microbes, which can make the host distinct from surrounding free-living microbiomes such as seawater (Glasl et al. 2019). Macroalgae can release nutrient rich exudates and polysaccharides, which can form micro-niches that promote certain bacterial lineages (Martin et al. 2014, Singh and Reddy 2014, Vieira et al. 2016b, Morrow et al. 2017). The release of secondary metabolites and organic compounds by macroalgae also may promote proliferation of heterotrophic bacteria (Haas et al. 2016, Roach et al. 2017). These exudates provide a food source for bacteria, thus macroalgae can act as an ideal micro-niche for these bacteria to survive (Barott and Rohwer 2012). Bacteria belonging to the family *Saprospiraceae* were abundant across both *Sargassum* species, and bacteria within this family have been linked to the breakdown of complex macro-molecules such as polysaccharides from exudates (McIlroy and Nielsen 2014). Furthermore, bacteria within the class *Gammaproteobacteria* and associated families (e.g. *Alteromonadaceae* and *Woeseiaceae*) were dominant in both *Sargassum* species. *Gammaproteobacteria* are common in marine environments (Cho and Giovannoni 2004), particularly within nutrient rich niches such as marine biofilms (Wietz et al. 2010, López-Pérez and Rodriguez-Valera 2014, Franco

et al. 2017). Sequences associated with *Alteromonadaceae, Woeseiaceae* and *Saprospiraceae* were not retrieved from seawater samples, and highlight potential bacteria that may be associated specifically with the *Sargassum* host.

Through the production of nutrient rich exudates, not only can the *Sargassum* host provide a niche for certain bacterial assemblages to colonise, but the bacteria in turn may offer advantageous characteristics for host survival. For example, bacteria belonging to the family *Saprospiraceae*, which were found in abundance across both *Sargassum* species and all regions, have been observed feeding on pathogenic and opportunistic bacteria (Aizawa 2005, Shi et al. 2006). As such, *Sargassum* species may be host to bacteria that can offer a defensive mechanism against potentially harmful microbes, which could contribute to sustaining host health. Furthermore, bacteria belonging to the family *Rhodobacteraceae* were dominant across both species of *Sargassum*, and many marine taxa within this family have been linked to sulphur and carbon biogeochemical cycling (Pujalte et al. 2014). The release of different nutrients as products from biogeochemical cycling by the bacteria are then made available for other organisms and the bacteria-associated host to utilise the nutrients for metabolic function (Fiore et al. 2010). As such, some of the bacteria found to be associated with the *Sargassum* species within this study may provide an important nutrient source to maintain host health. Whilst such bacteria-host benefits can be inferred, there remains limited discussion around the roles and functions of the macroalgae microbiome. Further functional metagenomic and metstranscriptomic work is essential in order to elucidate the functions particular bacteria may have in relation to maintaining host fitness.

2.5.2 Microbial communities differ across *Sargassum* regions

Microbial communities associated with macroalgae have predominantly been characterised from algal-associated biofilms (Kanagasabhapathy et al. 2006, Lachnit et al. 2011, Egan et al.

2013, Martin et al. 2014, Dogs et al. 2017), rather than different algal tissues such as the leaf, primary axis (stem) and holdfast. This current study observed significant differentiation in the bacteria communities of algal tissues from different regions of *Sargassum*. Within this study, niche clustering was observed; the biofilm of both *Sargassum* species was significantly more diverse than other regions of the alga, and the most abundant bacterial taxa of the leaf and stem was similar, as was the basal growth and holdfast. Due to distinct diversity clustering, and to simplify discussion of bacterial community differentiation, the biofilm, leaf and stem are referred to as 'upper regions', and basal growth and holdfast 'lower regions'.

In terrestrial environments, plant morphological attributes can drive the structure and assembly of microbial communities (Turner et al. 2013, Wallace et al. 2018), for example, root-associated bacterial communities are different to those from the leaf (Bodenhausen et al. 2013, Fitzpatrick et al. 2018). Similarly, upper and lower region differentiation was observed across both *Sargassum* species, with the overall diversity of the lower regions of *Sargassum* species appearing more variable than the upper regions. Relative abundance of bacteria belonging to the Phylum *Firmicutes* was highest in the basal growth and holdfast of both *Sargassum* species. Bacteria belonging to the ASV *Geobacter* was only found in the lower regions of *S. aquifolium*. Within marine environments, bacteria belonging to *Firmicutes* have been associated with marine sediments and benthos (da Silva et al. 2013), and *Geobacter* has been recognised in association with anaerobic respiration in aquatic sediments (Lovley et al. 2011, Ueki et al. 2018). The holdfast and basal growth of *Sargassum* species generally live covered by marine sediment and other benthos material in anaerobic conditions (Umar et al. 1998, Kawamata et al. 2012), hence it is probable this may influence the abundance of retrieved *Firmicutes* and *Geobacter* sequences within these lower regions.

Marine microbes that colonise coral reef organisms potentially offer functional benefits to the host (Neave et al. 2017, van de Water et al. 2018, Shiu and Tang 2019). Sequences of the ASV *Bradymonadales* (Wang et al. 2015) were only retrieved in the leaf and biofilm of both algal species. This particular ASV was found to be associated with macroalgae (Aires et al. 2016), and bacteria belonging to *Bradymonadales* in marine environments have been proposed to aid sulphur-cycling within marine environments (Wang et al. 2015). Furthermore, bacteria such as *Loktanella* and *Saprospiraceae,* whilst present across all *Sargassum* regions, were in highest abundance on the leaf and biofilm of *Sargassum,* and both bacteria have potential functional roles in nutrient cycling, for example, making nutrient available to the host for metabolic processes (Miranda et al. 2013, McIlroy and Nielsen 2014, Tanaka et al. 2014, Ma et al. 2017). The higher abundances of these bacteria on the upper regions of *Sargassum* may assist in nutrient provision to the alga, but as this was the result of 16S data, further microbial functional analysis is essential to understanding functional properties of *Sargassum* associated bacteria.

2.5.3 A diverse microbial assemblage is observed on the *Sargassum* leaf and biofilm

Differentiation between the *Sargassum* regions was characterised across both *Sargassum* species, although the leaf and biofilm in particular were observed to host a highly diverse microbial community. Scanning electron microscopy of the *Sargassum* leaf visualised the alga's biofilm and revealed a wide range of cell structures present on the surface. In particular an extensive array of diatoms and bacteria were observed. These microorganisms are among the first colonisers of surfaces in marine environments (Cooksey and Wigglesworth-Cooksey 1995), playing an important role in the formation of marine biofilms and biogeochemical activity within the biofilm (Patil and Anil 2005, Lage and Graça 2016). Both diatoms and bacteria secrete extracellular polymeric substances (EPS), generally comprised of

polysaccharides, lipids and humic substances, which help form biofilms (Underwood and Paterson 2003, de Carvalho 2018).

Many organisms such as macroalgae can provide a source of nutrients for planktonic diatoms and bacteria to attach to and produce biofilms (Horváthová et al. 2016). Biofilms on macroalgae surfaces comprised of macro molecules and organic compounds can become sources of nutrients for higher trophic level organisms (e.g. isopods and detritovores) (Chiu et al. 2007, Horváthová et al. 2016). In turn, EPS secreted by diatoms and bacteria can provide some defences to the host surface (e.g. macroalgae), such as protection against pH and temperature fluctuations, UV exposure, salinity changes, and depletion of nutrients (de Carvalho 2018). Bacteria have also been attributed to providing essential antifouling properties to protect macroalgae from secondary colonisation from pathogenic microbes (Egan et al. 2013). Specifically, within this study, SEM revealed a variety of pennate and centric diatoms, and potential coccoid bacterial clusters and prosthecate filaments associated with bacteria cellular membranes. Furthermore, the use of FISH confirmed the presence of bacteria across the *Sargassum* leaf surface. The images from FISH and SEM highlight diatoms and bacteria are fundamental structures within the *Sargassum* biofilm, and although the exact function of diatoms and bacteria observed cannot be attributed, the presence of a diverse biofilm community on the *Sargassum* surface may contribute to the physiological function and health of the macroalgae. Microscopy has proven a useful descriptive tool for exploring the baseline microbial and microscopic communities of the *Sargassum* biofilm. Future studies using microscopy to investigate the structure of macroalgae surfaces should consider in depth identification of diatoms present and bacteria specific FISH probes to further explore the functional microbe-macroalgae relationship.

Potential fungal morphologies were visualised using SEM. Fungi, like bacteria, have been associated with cycling organic matter in marine ecosystems and Antarctic macroalgae (Loque et al. 2010). Specifically within terrestrial plants, endophytic fungal communities have been associated with utilising photosynthetic nutrients (Marcial Gomes et al. 2003, Zhang and Yao 2015), and fungi are primarily responsible for the transformation of plant-derived carbon in terrestrial ecosystems (Voříšková et al. 2014). As potential fungal morphologies were observed on the *Sargassum* leaf surface, and fungal communities have an apparent nutrient cycling function within terrestrial plants, it is essential that future work explores the presence and distribution of fungal communities across macroalgae to capture all microbial components of the macroalgae microbiome.

The leaf and biofilm of both *Sargassum* species hosted the most abundant and diverse bacterial communities of all the regions that most likely offer important roles in maintaining host health. Generally, macroalgae leaves provide the largest surface area for biofilm to form (Martin et al. 2014), which contributes to macroalgal leaves forming diverse bacterial niches (Cooksey and Wigglesworth-Cooksey 1995). Whilst present across both *Sargassum* species' regions, bacteria belonging to the family *Rhodobacteraceae* were most abundant within the leaf and biofilm, in particular the genus *Loktanella*. *Loktanella* has been previously described in association with seawater, marine sediments and biofilms (Lau et al. 2004, Tanaka et al. 2014, Ma et al. 2017). Serebryakova et al. (2018) revealed a high presence of *Loktanella* in endophytic and epiphytic microbial communities of *Sargassum muticum*, and previous bacterial community sequencing of the alga *Porphyra umbilicalis* revealed *Loktanella* are core taxa within the alga's microbiome (Miranda et al. 2013). *Loktanella* are phototrophic, involved in sulphur and carbon biogeochemical cycling and symbiosis of aquatic micro- and macro-organisms (Pujalte et al. 2014). The release of different nutrients as products from biogeochemical cycling are then made available for other organisms to utilise for metabolic function (Fiore et al. 2010). Thus,

it is likely that *Loktanella* may be a physiologically important taxa for macroalgae function and health.

FISH analysis of the *Sargassum* leaf revealed various bacterial structures across the leaf surface. In particular, coccoid-like epiphytic bacterial clusters and filamentous structures. Interestingly, some bacteria belonging to the family *Saprospiraceae* have been recorded to have a filamentous structure (McIlroy and Nielsen 2014), and within this study *Saprospiraceae* was found to be most abundant within the leaf and biofilm of both *Sargassum* species. The family *Saprospiraceae* have previously been found to be an abundant bacteria associated with *Sargassum* (Serebryakova et al. 2018), and other macroalgae species such as *Asparagopsis* species (Aires et al. 2016). Bacteria within the *Saprospiraceae* family have been linked to the breakdown of complex biological and organic macromolecules, in particular polysaccharides and proteins, through an observed capacity for hydrolysis (Burke et al. 2011, McIlroy and Nielsen 2014). Such traits are thought to assist in providing effective metabolic function and nutrient cycling to the host that the bacteria is associated with (Kirchman 2002). Furthermore, *Saprospiraceae* related organisms are rarely present as free-living organisms in marine environments, instead attaching to surfaces rich in complex nutrient sources such as epibacterial communities and biofilms of macroalgae (McIlroy and Nielsen 2014, Lage and Graça 2016). From this study, we found abundances of *Saprospiraceae* bacteria across all *Sargassum* regions, with highest concentrations within the leaf and biofilm of both *Sargassum* species. Thus, the traits of the *Saprospiraceae* family described are the most likely explanation for why this particular bacteria family is so abundant within *Sargassum* and in particular the epiphytic niches.

This study provides a baseline dataset of the bacteria communities present within *Sargassum* species at Magnetic Island (GBR), to enable further study the role of macroalgae associated

microbes in coral-algal interactions. The leaf and biofilm of the *Sargassum* species displayed the highest diversity of all the regions, and when considering direct interactions between coral and fleshy macroalgae, these components can be the first point of contact between coral and *Sargassum*.

To study the direct interactive effect of *Sargassum* on the coral microbial community, the biofilm in particular could be considered an important component in assessing the role of microbes in coral-algal interactions. While previous studies examining the macroalgae microbiome have largely focused on sequencing the macroalgae biofilm alone, this study reveals *Sargassum* has a diverse bacterial community that differs across different regions of the alga that may underpin host fitness. Therefore, future research investigating the microbial assembly of macroalgae should consider examining host tissues alongside biofilm communities with functional analysis undertaken to decipher links between bacterial assemblies present and host fitness.

2.6 Conclusions

This study acts as an important baseline step in characterising the bacteria that are present in tropical variants of *Sargassum* species. These results can inform future research regarding the role *Sargassum* associated microbes may have in influencing the surrounding environment on coral reefs. Importantly, whilst the microbiome between the *Sargassum* species investigated was similar, there was microbial distinction between alga's regions. As apparent region based niches were observed across *Sargassum* species, it is key that future research explores morphologically separated microbial assemblies when investigating the factors that make macroalgae a successful competitor on coral reefs. To address the overall aim of this thesis, it was concluded the biofilm would be used to explore direct microbial coral-algal interactions between *Sargassum* species and coral. When *Sargassum* is in direct contact with coral, the

biofilm covering the algae is the first component to touch the coral. Furthermore, the *Sargassum* biofilm was found to host numerous microbial structures and a diverse bacterial community.

3 Microbial coral-algal interactions on the inshore Great Barrier Reef

3.1 Abstract

Coral-algal interactions can have detrimental effects on coral contributing to decline in reef health, which may be partly driven through direct and indirect microbial processes. Within this study, 16S rRNA gene amplicon sequencing was conducted on *Sargassum* species and *Montipora aequituberculata* to assess how direct contact between the two species affects host bacterial communities. *Sargassum* and *M. aequituberculata* samples were collected from Magnetic Island (Central GBR) from different proximity treatments (isolation, direct contact and systemic proximity) and across three time points (December 2017, February 2018 and May 2018) to explore how the host bacterial communities may change over time and in relation to proximity interactions. Benthic community surveys revealed significantly higher *Sargassum* abundances in February 2018 (43.3% +/- 10) compared to May 2018 (14.2% +/- 2.4). Analysis of the 16S rRNA data revealed the *Sargassum*, *M. aequituberculata* and seawater bacterial community compositions were all significantly different from each other. Across proximity treatment and time, the *M. aequituberculata* community was dominated by sequences affiliated with the bacterial families *Pirellulaceae*, *Nitrosopumilaceae*, *Rhodobacteraceae* and *Flavobacteriaceae*. *Sargassum* species bacterial community was dominated by sequences associated with *Flavobacteriaceae*, *Rhodobacteraceae* and *Saprospiraceae*. Both the *Sargassum* and *M. aequituberculata* bacterial communities displayed distinct temporal shifts. Sequences affiliated with *Saprospiraceae* (commonly affiliated with macroalgae) in the *Sargassum* bacterial community were highest in February 2018 compared to May 2018, most potentially linked to changes in *Sargassum* density between February and May. Whilst the

diversity and community assemblage of *Sargassum* and *M. aequituberculata* bacterial communities remained consistent across proximity treatments, some shifts in the community structure of direct contact samples were observed. For example, sequences associated with the bacteria family *Endozoicomonadaceae*, previously implicated as highly abundant in healthy corals, were most prevalent within *M. aequituberculata* isolation samples and lowest in direct contact samples, especially in February 2018 when *Sargassum* abundance was highest. Stability of the *M. aequituberculata* bacterial community in direct contact with *Sargassum* indicates that micro-scale coral-algal interactions appear to have little impact on the adult *M. aequituberculata* host microbial community at Magnetic Island. This may be reflective of the tolerance of this species, and explain its ecological success despite the conditions and interactions the reefs are exposed to at Magnetic Island. Further research should investigate whether this stability is consistent across other coral species, especially more sensitive branching acroporids, and if it can be replicated on other inshore reefs that have had less historical exposure to *Sargassum*.

3.2 Introduction

Within marine environments, competitive interactions between species can promote the survival of one organism over another (Buss and Jackson 1979, Aerts and Van Soest 1997, Kuffner et al. 2006, Forrester 2015). One of the most significant competitive interactions on coral reefs exists between macroalgae and coral (Hay 1997, McCook et al. 2001, Swierts and Vermeij 2016, O'Brien and Scheibling 2018). Primarily, macroalgae and coral compete for space for propagation and light for photosynthesis (McCook et al. 2001, Vroom et al. 2006, Box and Mumby 2007). While the broad effects of these interactions have been investigated at the macro-scale, there remains limited understanding on how macroalgae impact coral at a microbial scale. Microorganisms respond rapidly to altered environments conditions, in part due to short generational turnaround, and thus microbial communities can become destabilised,

subsequently influencing overall fitness of the coral holobiont (Schimel etl al. 2007, Egan et al. 2013, Bourne et al. 2016, Rocca et al. 2019). Thus, it is critical to consider how direct coral-algal interactions influence the microbial communities of both host species.

Increase in macroalgal abundance on reefs can have direct negative impacts on corals through shading and abrasion (Smith et al. 2006, Birrell et al. 2008a, Ceccarelli et al. 2018) and by changing the coral microbiome (Rasher and Hay 2010, Barott and Rohwer 2012, Leong et al. 2018). For example, Barott et al. (2012) observed tissue abrasion and necrosis of *Montastrea annularis* where turf algae grew attached to the coral, while Diaz-Pulido et al. (2009) noted localised bleaching on *Acropora sp.* where *Lobophora sp*. that had been growing on the coral had been removed. Microbial communities derived from algae can also impact coral health, with Vieira et al. (2016) detecting areas of localised bleaching of *Acropora* species within 24 h when bacteria isolated from *Lobophora* species were applied to the coral surface. As macroalgae abundance increases, opportunity for direct contact and thus direct microbial interactions between macroalgae and coral also increases. Throughout a thermal stress event, which promoted growth of turf macroalgae over corals, Pratte et al. (2018) witnessed increased similarity between the microbiome of *Porites* and turf macroalgae at points where the two organisms were in direct contact, suggesting a transfer of microbes. Furthermore, direct microbial coral-algal interactions can have macro-scale impacts (Brown et al. 2018, Clements et al. 2018). Barott et al. (2012) proposed a model whereby macroalgae can act on a micro-scale to stress corals, leading to macro-scale changes to reef ecology. For example, contact between turf algae and *Montastrea annularis* eliminated oxygen production at the contact interface (Barott et al. 2012), contributing to reduced coral growth (Lirman 2001, Barott et al. 2009), lower coral fecundity (Foster et al. 2008) and the inhibition of coral larval settlement (Birrell et al. 2005).

Indirect coral-algal interactions can also influence coral health, primarily through reduced space for coral propagation (Kuffner et al. 2006, Brown et al. 2018, Leong et al. 2018). Moreover, indirect mechanisms can also influence the coral microbiome through release of algal exudates such as dissolved organic carbon (DOC) (Smith et al. 2006, Hauri et al. 2010, Haas et al. 2016). This indirect mechanism has been summarised within the Dissolved organic material, Disease, Algae and Microbes (DDAM) model; increased macroalgae abundance promotes the release of bioavailable DOC into the environment, initiating microbialisation of the reef ecosystem (Barott and Rohwer 2012, Haas et al. 2016). Microbes can outcompete corals for bioavailable resources, depriving corals of the necessary nutrients (e.g. carbon and nitrogen) needed for survival, leading to a higher chance of coral degradation (Barott and Rohwer 2012, Haas et al. 2016, Roach et al. 2017), which can in turn promote increased algal cover (Smith et al. 2006).

Environmental stress can also disturb the stability of coral associated microbes (Rohwer et al. 2002, Thurber et al. 2009, Sunagawa et al. 2010). Coral bleaching, for example, can shift the bacterial community structure of corals (Bourne et al. 2008), and increased nutrient inputs can trigger coral disease lesions, which can destabilise healthy coral microbiomes (Bourne 2005, Rosenberg et al. 2007). Similarly, changes have also been recorded in the macroalgae microbiome as a result of environmental change (Egan et al. 2013, Zozaya-Valdés et al. 2017, van der Loos et al. 2019). For example, bleaching and a reduction of bacterial diversity in the temperate algae *Delisea pulchra* throughout periods of higher seawater temperatures have been documented (Campbell et al. 2011). Previous studies investigating the nature of coral-algal interactions have focused on macro-scale interactions and the different ways in which they may impact the coral itself and surrounding environment (Mumby 2009, Hoey and Bellwood 2011, Lefévre and Bellwood 2011, Bruno et al. 2015). Consequently, as macroalgae continues to proliferate on coral reefs and there are clear links between shifts in microbial and ecosystem

health, understanding microbial coral-algal interactions is integral to help identify which factors may promote algal dominance over corals, and how this may exacerbate decline in coral health.

To date, research investigating the coral-algal interactions on corals of the GBR have examined the effects of allelochemicals produced by the brown alga *Lobophora* on coral larvae and associated microbial communities of *Porities* coral (Morrow et al. 2017). *In situ* field tests detailing microbiome shifts of algae and coral hosts as a result of direct interactions, however, have not been conducted. As the frequency and magnitude of disturbance events increases on the GBR (Hughes et al. 2017), there is increasing risk of higher macroalgal dominance on some reefs (Dudgeon et al. 2010), with the risk of increased algal abundance highest on inshore reefs of the GBR due to the proximity of coastal development and elevated sediment and nutrient inputs (Fabricius 2005, Fabricius et al. 2005). Magnetic Island (inshore Central GBR) has seen an increase in macroalgae cover following disturbance-driven declines of hard coral cover (Ceccarelli et al. 2019). Furthermore, significant negative relationships between coral and macroalgae abundance have been observed at Magnetic Island, especially once macroalgae cover exceeds 20% (Ceccarelli et al. 2019). Algal abundances change on a seasonal scale at Magnetic Island, with rapid growth throughout the Austral summer (Martin-Smith 1992, Brown et al. 2018). Nonetheless, despite seasonally prolific algae and low abundances of macroalgae grazing fish (Ayling and Ayling 2005), relatively stable hard coral cover (though low diversity community composition) exists on the reefs around Magnetic Island (Ceccarelli et al. 2019). The mixed community of coral and macroalgae therefore provided an ideal location to study the influence of direct coral-algal interactions on their respective host's microbial communities.

The overall aim of this chapter is to investigate micro-scale coral-algal interactions, specifically combining microbial analysis and benthic community surveys at Magnetic Island (Geoffrey Bay) to investigate the effect of direct contact between *Sargassum* species and *M. aequituberculata* on their respective bacterial communities, including how this changes over the seasonal growth cycle of *Sargassum* species (Martin-Smith 1992). The benthic community surveys were completed by assessing the percent cover of *M. aequituberculata* and the density of *Sargassum* species to quantify how the benthic assemblage changed over time. 16S rRNA gene amplicon sequencing was used to investigate microbial changes between *M. aequituberculata* and *Sargassum* species in varying proximities to each other and over time.

3.3 Methods

3.3.1 Study site and species

The study site was Geoffrey Bay, Magnetic Island (19^{o} 09’ 09.90” S, 146^{o} 52’ 03.28” E) with further details of this site presented in Chapter 2. *Montipora* species were the most abundant coral present in 2017, accounting for more than half of the total coral cover (AIMS, 2019). *Montipora aequituberculata* was selected due to its plating morphology facilitating ease of sampling. *Sargassum* species follow seasonal growth trends (Martin-Smith 1993, Schaffelke and Klumpp 1997) and account for more than half of the total macroalgae cover at Magnetic Island (AIMS, 2019). The natural growth and senescence cycle of the alga acts as a proxy for fluctuating macroalgae abundance over the seasonal sampling regime. Sample collections and surveys took place at three time points (4th December 2017, 13th February 2018, and 25th May 2018). At each time point, coral, macroalgae and seawater samples were collected for microbial community analysis and benthic assemblages were quantified to describe how the sampling environment changes over time (see section 3.3.2). As little distinction between the two *Sargassum* species microbiomes was observed in Chapter 2, it was decided the *Sargassum*

samples for Chapter 3 would not be identified to species level, and the interaction study focusses on the genus *Sargassum* and *M. aequituberculata* at Geoffrey Bay (Magnetic Island).

3.3.2 Benthic community surveys

Point intercept transects were completed at Geoffrey Bay, Magnetic Island alongside sample collection to quantify any changes in benthic assemblages over time. Three replicate 20m transect tapes were randomly laid across the site at approximately 5-7m depth. Every 50cm along each transect the substratum directly below the tape was recorded as coral, algae, rock, sand or rubble. Corals and algae were identified to genus and species where possible.

Quadrat surveys were used to monitor how the density and canopy height of *Sargassum* species, and the amount of contact between *Sargassum* species and *M. aequituberculata* changed over time between sampling time points. Six 1 x 1m quadrats were randomly placed across the study site to quantify the benthic assemblage. Within each quadrat, the following observations were recorded: percent cover of *Sargassum* species; percent cover of *M. aequituberculata*; percent contact between *Sargassum* species and *M. aequituberculata*; percent of *M. aequituberculata* diseased/lesions present; number of *Sargassum* species holdfasts; and *Sargassum* species canopy height. *Sargassum* canopy height was quantified by measuring the height of three randomly selected *Sargassum* thalli from holdfast to tip and calculating the mean height. Contact between *Sargassum* species and *M. aequituberculata* was measured by estimating the percent of *M. aequituberculata* completely smothered by *Sargassum* within each quadrat. *M. aequituberculata* colonies in each quadrat affected by disease or lesions were counted, and the tissue area of each colony estimated within categories (0-25%; 26-50%; 51-75% and 75-100%). This area was normalize over total coral colony tissue area to provide an inference for allelopathic activity (Table S2).

3.3.3 Benthic community analysis

ANOVA models were constructed to determine if percent cover estimates of *Sargassum* species and the percent contact between *Sargassum* species and *M. aequituberculata* changed over time. The number of *Sargassum* holdfasts and the *Sargassum* canopy height were measured as a proxy for *Sargassum* density, and were similarly modelled using ANOVA to investigate the differences in the number of holdfasts and canopy height over time. Tukey post-hoc pairwise comparisons were conducted to establish differences between individual time points for each ANOVA model. All models were fitted using R v3.5.3 (R Core Team, 2018) and pairwise comparisons conducted using the "lsmeans" package (Lenth 2016). Model assumptions (normality and homogeneity of variances) were visually inspected via boxplots and residual plots.

3.3.4 Sample collection

Samples were collected from *M. aequituberculata* and *Sargassum* species that were isolated from each other (>5m away), in direct contact (area of the organisms touching), and in systemic proximity (the same organisms in contact, but sampled ~10cm away from the point of direct contact) (Figure 3.1, Figure 3.2). Approximately 15-20cm pieces of *Sargassum* frond and 5 cm x 5 cm pieces of *M. aequituberculata* were collected from each proximity treatment. 5 replicates of each proximity treatment of both *Sargassum* and *M. aequituberculata* were collected per time point (*Sargassum* time point n = 15; *M. aequituberculata* time point n = 15; *Sargassum* total n = 45; *M. aequituberculata* total n = 45). *Sargassum* samples were collected using sterile gloves and sterile scissors, and *M. aequituberculata* samples were collected using sterile gloves and a sterile hammer and chisel. Samples were immediately rinsed in sterile artificial seawater, preserved in EDTA-DMSO (Table S1), and later stored at -20^{o}C. Seawater samples of 2 x 2Ls were collected at each sampling time point, filtered using 0.22µm Sterivex

filter (Merck Millipore), and stored using the same methods described previously (see Chapter 2, samples collected using permits G15/37574 and G16/38348).

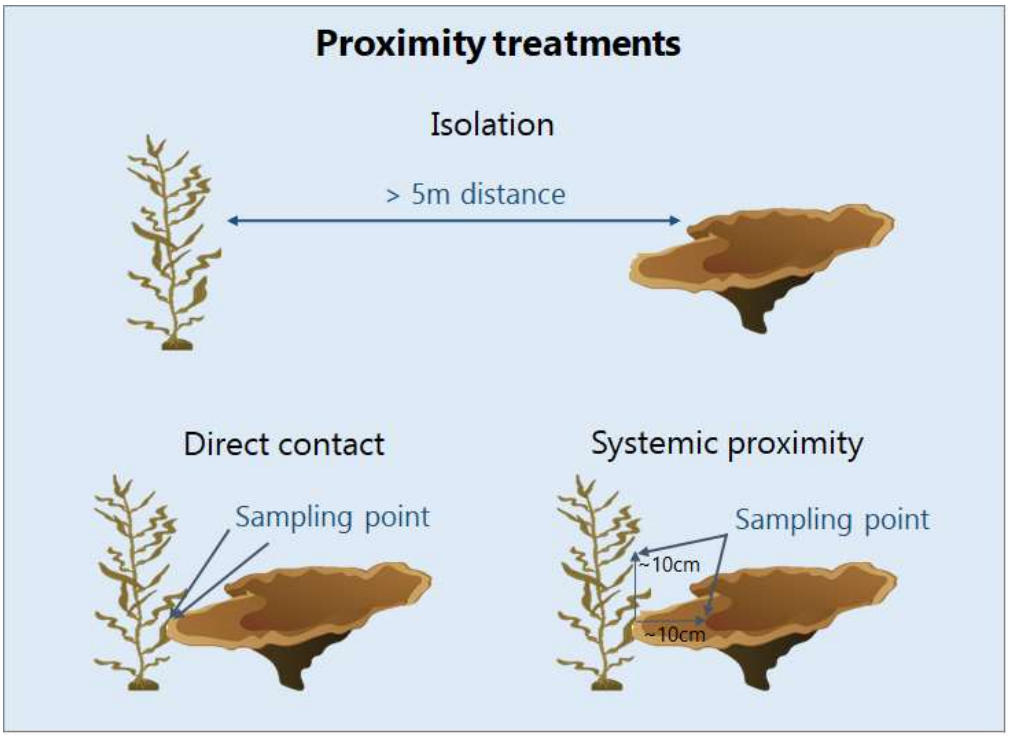


Figure 3.1 Proximity treatments to collect *Sargassum* and *M. aequituberculata* samples (icons from Tracy Saxby and Jane Thomas, IAN image library (ian.umces.edu/imagelibrary), figure credited to author).

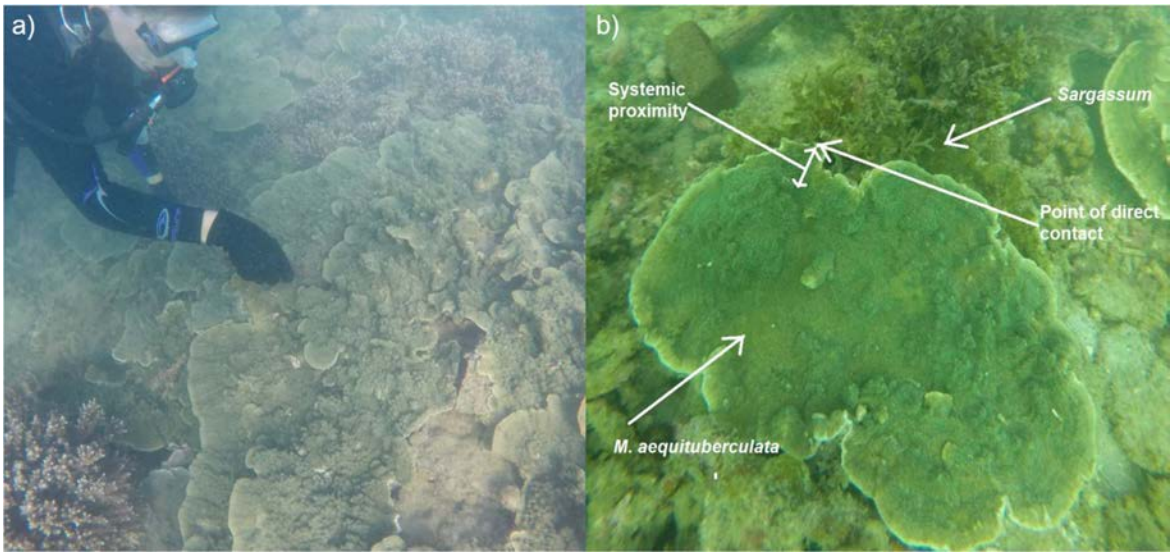


Figure 3.2 a) *M. aequituberculata* in isolation proximity (Geoffrey Bay, Magnetic Island). b) *M. aequituberculat*a and *Sargassum* species in direct contact, highlighting direct contact interface and systemic proximity (Geoffrey Bay, Magnetic Island. Photographed May 2018, credit to G. Al Moajil-Cole.

3.3.5 Total genomic DNA extraction

Prior to DNA extraction, *M. aequituberculata* samples were tissue blasted in 5 mL of sterile artificial seawater to remove tissue and mucus. 2 mL of the coral tissue slurry was then aliquoted into centrifuge tubes and centrifuged at 10,000 x g for 10 min. DNA was extracted from the resulting pellet and the supernatant discarded. DNA was extracted from the coral pellet using the *Qiagen DNeasy PowerBiofilm Kit* following the manufacturer's instructions with the exception of the same alterations used during DNA extraction in Chapter 2 (Section 2.2.2). *Sargassum* DNA was extracted from the biofilm, which was removed from the *Sargassum* samples using the same methods described in the previous chapter (Section 2.2.1). *Sargassum* DNA was extracted from the biofilm also using the *Qiagen DNeasy PowerBiofilm Kit* using the same modifications and methods from Chapter 2 (Section 2.2.2). DNA was extracted from the Sterivex filters using the same methods detailed in Chapter 2 (Section 2.2.2).

3.3.6 PCR and 16S rRNA gene amplification

The V4 region of the 16S rRNA gene of *Sargassum*, *M. aequituberculata* and seawater was amplified used 515F (modified) (5' – GTG YCA GCM GCC GCG GTA A – 3') and 806R (modified) (5' – GGA CTA CNV GGG TWT CTA AT – 3') primers. Different primers were used in Chapter 3 compared to Chapter 2 as 515F and 806R primers are commonly used for amplification of the 16S rRNA gene in corals. Each primer was fused with the forward and reverse Illumina overhang sequences (forward overhang: 5'–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG–3', reverse overhang: 5'–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG–3') (Illumina 2013). The following amplification protocol was used: after an initial denaturation at 95°C for 1min, conditions were 35 cycles of denaturation at 95°C for 15s, annealing at 55°C for 15s, and extension at 72°C for 15s. The final extension was at 72°C for 15min. The 50 µL reaction contained 1 µL of each primer (20 µM), 25 µL MyFi 2X Mix (Bioline), 21 µL Milli-Q water and 2 µL template DNA

(1:10 dilution). The PCR product was submitted to Ramaciotti Centre for Genomics, UNSW Sydney for bead purification, library preparation (using Illumina MiSeq DNA library preparation protocol) and paired-end (2x250) Illumina MiSeq sequencing.

3.3.7 16S rRNA analysis and bacterial community diversity

Sequence reads of all samples were processed using QIIME2 (Caporaso et al. 2010). Sequence reads returned from sequencing demultiplexed. Reads were filtered and rarefied for quality and chimeric sequences using DADA2 (Callahan et al. 2016). Taxonomic classification was assigned using a naïve Bayes classifier, trained on the extracted regions of interest from the SILVA 16S rRNA (99) reference alignment (132 QIIME release (Quast et al. 2013)). All sequences classified as chloroplast, mitochondria or Eukaryota were removed. The resulting amplicon sequence variant (ASV) table was used for statistical analysis in Calypso (Zakrzewski et al. 2016) and R Studio (R Core Team 2017).

Alpha diversity was calculated using Shannon's diversity index and Faith's richness index. Patterns in microbial community composition were visualized using non-parametric multidimensional scaling (NMDS). Permutational multivariate analysis of variance (PERMANOVA) was used to identify differences in the microbial community composition of *Sargassum, Montipora* and seawater as a function of proximity treatment and time. The percent relative abundance of each microbial phylum and family present across *Sargassum* species, *M. aequituberculata* and seawater was calculated, and the most abundant bacterial taxa were plotted to analyse how the bacteria community changes over time and with proximity between coral and algae. ASV analysis of the most abundant bacterial taxa associated with *Sargassum* species, *M. aequituberculata* and seawater were identified down to the lowest possible taxonomic level, and BLASTed against the NCBI database.

3.4 Results

3.4.1 Benthic community analysis

An interaction between the percent cover of *Sargassum* species and sampling month was observed (Figure 3.3a). The mean percent cover of *Sargassum* species was higher in December 2017 (25.8% +/- 7.7) and February 2018 (43.3% +/- 10.0) compared to May 2018 (14.2% +/- 2.4). The percent cover of *Sargassum* species varied over time (ANOVA; $F_{df=2,15}$ = 3.94, p=0.042) and was found to be significantly lower in May 2018 compared to February 2018 (Tukey; $t_{df=15}$ = 2.78, p=0.035). The mean percent cover of *M. aequituberculata* across sampling time points was 31.7% (+/- 5.8).

Similar seasonal patterns were also observed for *Sargassum* canopy height (Figure 3.3c). Canopy height varied over time (ANOVA; $F_{df=2,15}$ = 4.20, p=0.036), again showing significant declines from February to May (Tukey; $t_{df=15}$ = 2.79, p=0.034). The number of *Sargassum* holdfasts also varied over time, with significantly lower numbers in December compared to May (Figure 3.3b: Tukey; $t_{df=15}$ = -3.12, p=0.018). This is likely to be because counting holdfasts at the peak of *Sargassum* biomass is more difficult and more potentially prone to human error, rather than the number of holdfasts increasing across sampling periods.

Finally, the percent contact between *Sargassum* species and *M. aequituberculata* (described as the percent of *M. aequituberculata* completely smothered by *Sargassum* species within each quadrat) was monitored over time to assess how the abundance of *Sargassum* influenced the amount of contact between the two organisms (Figure 3.3d). While a similar pattern of seasonal fluctuation emerged, it was only significant at the 90% confidence level (ANOVA; $F_{df=2,15}$ = 3.12, p=0.074: Tukey February-May; $t_{df=15}$ = 2.26, p=0.094).

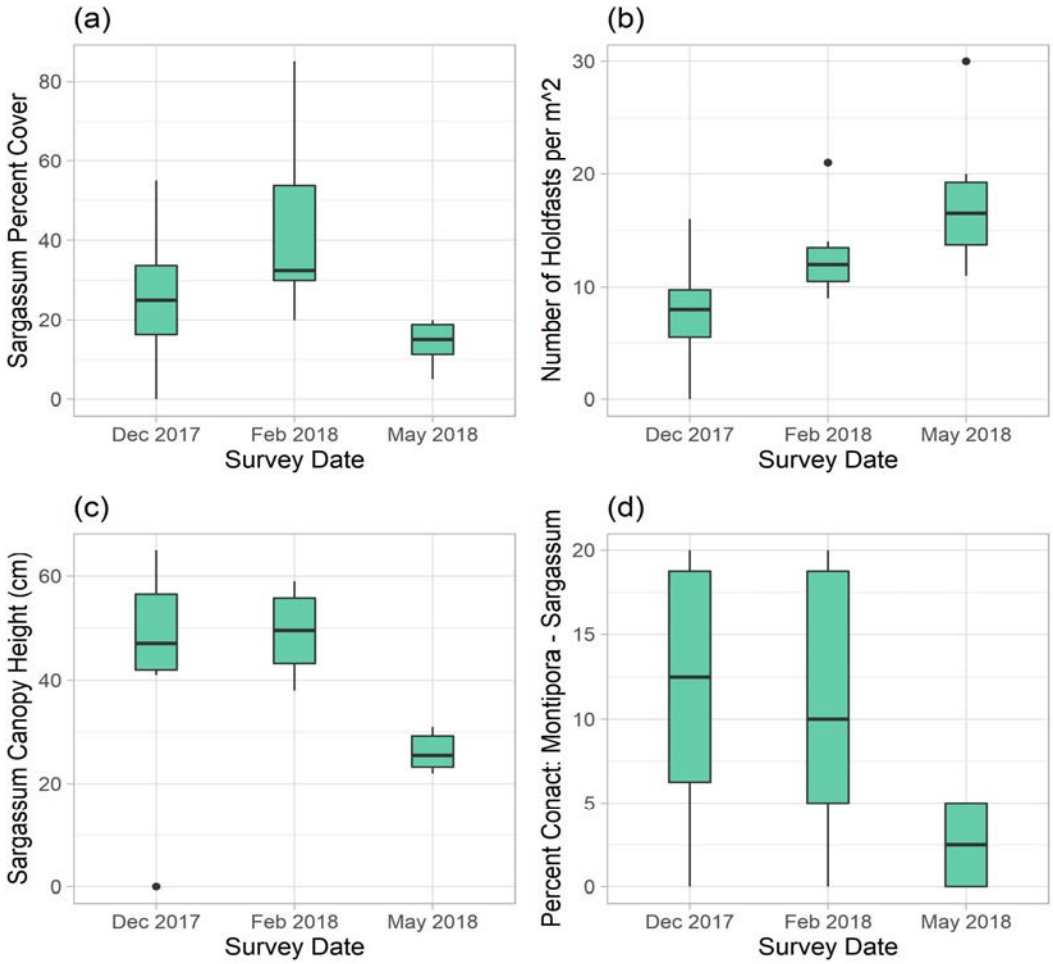


Figure 3.3 Analysis of benthic community assemblage variables, demonstrating how the density of *Sargassum* species fluctuated over time at the Geoffrey Bay study site. a) *Sargassum* species mean percent cover across sampling time point. b) The mean number of *Sargassum* species holdfasts across sampling time points. c) The mean *Sargassum* canopy height at the Geoffrey Bay study site across sampling time points. d) The percent contact observed between *Sargassum* and *M. aequituberculata* across sampling time point.

3.4.2 Bacteria community analysis

A total of 3,116,725 sequence reads were recovered from 96 samples derived from coral, macroalgae and seawater. Following filtering for high quality reads with 99% accuracy and removal of chimeric reads, a total of 844,110 high quality reads were subsequently used for taxonomic classification (Table S4). Rarefaction analysis confirmed sampling depth was sufficient to estimate the total diversity of each sample (Figure 3.4).

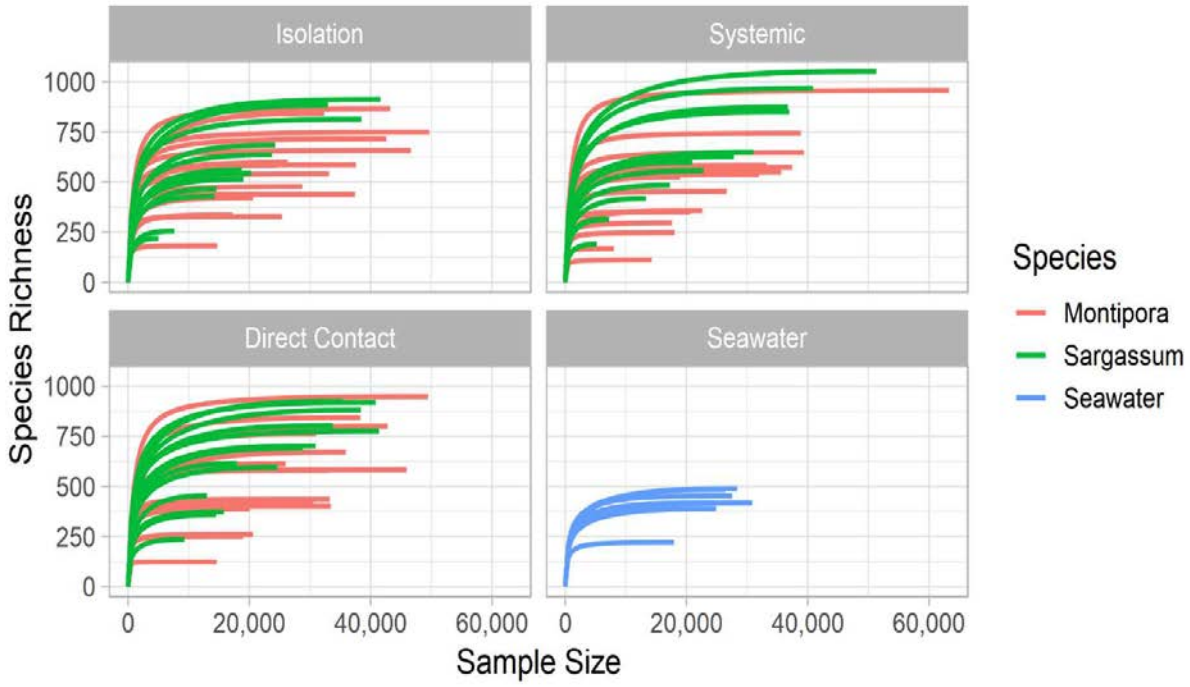


Figure 3.4 Rarefaction curves visualising sampling depth of each *Sargassum* species, *M. aequituberculata* and seawater samples grouped by proximity treatment.

3.4.2.1 *M. aequituberculata* bacterial community analysis

Diversity comparisons

Diversity analysis of the *M. aequituberculata* bacterial communities revealed there was a significant interaction between species and time. The diversity and richness of the December bacterial community was significantly lower than that of February and May (Faith's richness: ANOVA, $p = 0.0046$; Shannon's diversity: ANOVA, $p = 0.012$) (Figure 3.5a, b). No significant differences were observed in the bacterial community of *M. aequituberculata* across the three proximity treatments of isolation, direct contact and systemic (Shannon's diversity: ANOVA, $p = 0.83$; Faith's richness: ANOVA, $p = 0.99$) (Figure 3.5c, d).

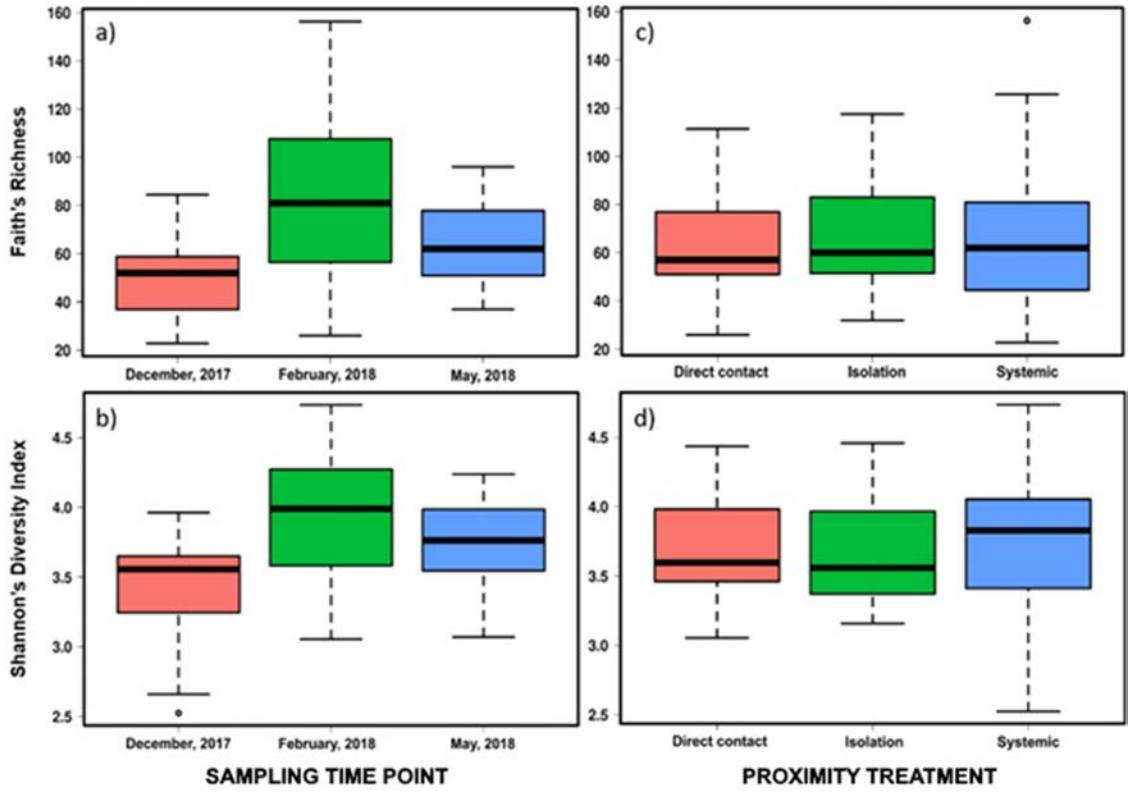


Figure 3.5 Alpha diversity of *M. aequituberculata* across sampling month and proximity treatment. Faith's richness (a) and Shannon's diversity (b) observed to be significantly lower in December compared to February and May sampling time points. No significant difference in the *M. aequituberculata* Faith's richness (c) and Shannon's diversity (d) across proximity treatment.

Bacterial community composition

Bacteria community composition analysis also showed there was a significant interaction between species and time. The *M. aequituberculata* bacterial community composition was significantly different between February and May (PERMANOVA: $R^2 = 0.156$, $p = 0.003$) (Figure 3.6a). No significant difference in the bacterial community composition of *M. aequituberculata* across proximity treatments was observed (PERMANOVA: $R^2 = 0.451$, $p = 0.46$) (Figure 3.6b).

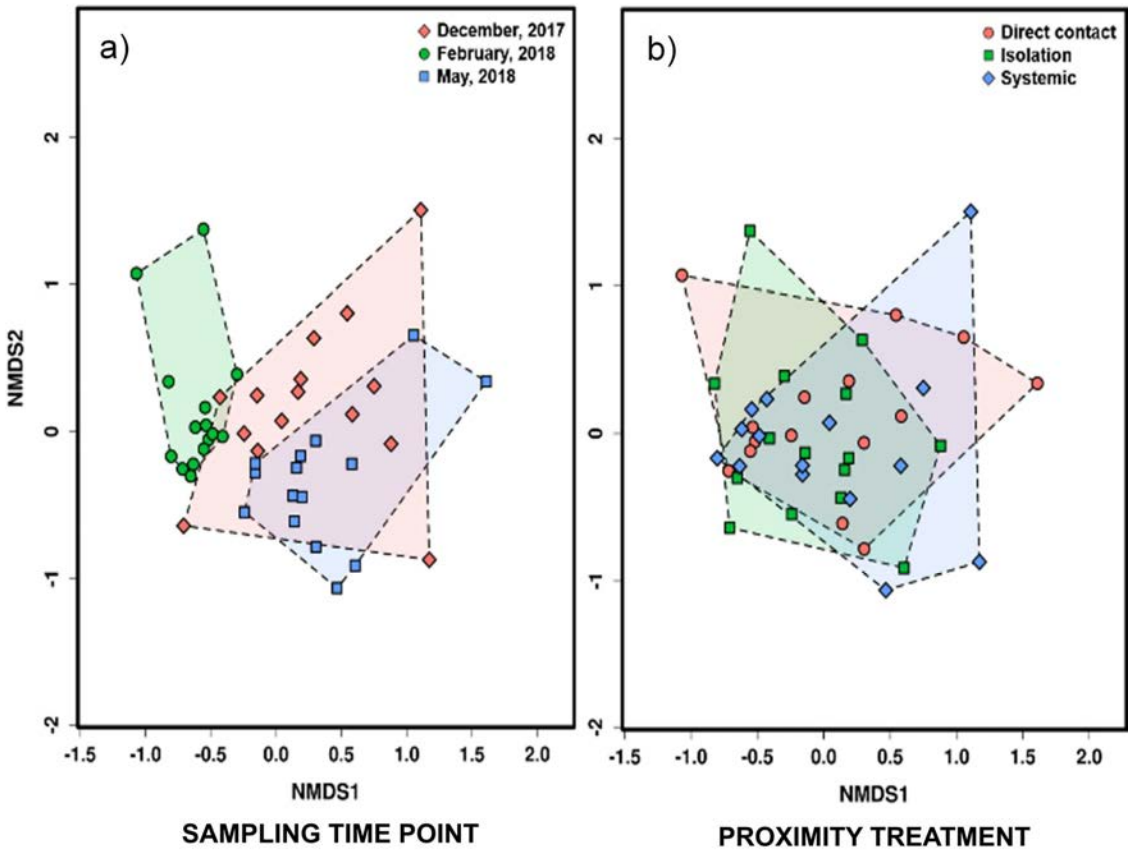


Figure 3.6 Bacteria community composition analysis of *M. aequituberculata*. a) February community composition of *M. aequituberculata* observed to be significantly different from the May sampling time point (December, red; February, green; May, blue). b) No changes in the *M. aequituberculata* microbial community observed across proximity treatments.

Sequences associated with 41 bacterial Phyla and 5 archaeal Phyla were identified from the 16S rRNA dataset derived from all *M. aequituberculata* samples. Archaeal phyla represented less than 2% of all retrieved sequences, and are therefore not discussed further. *Proteobacteria* affiliated sequences were the most abundant phyla, representing approximately 50% of all retrieved sequences (Figure S1, 2). There were no major changes in the relative abundance of sequences retrieved from *M. aequituberculata* proximity treatment samples (Figure S1), although small shifts in relative abundance of phyla were observed for corals sampled from the different time points (Figure S2). For example, *Proteobacteria* affiliated sequences were highest in relative abundance in December 2017 (47.4-54.8%) and May 2018 (50.7-53.1%), but represented only 44.8-47.0% of retrieved sequences in February 2018 (Figure S2).

Sequences affiliated with *Bacteroidities* were similarly highest in December (10.1-14.6%) and May (10.5-14.7%), and lowest in February (4.5-7.7%). By contrast, *Planctomycetes* displayed highest relative abundances in February 2018 (16.2-18.6%), but represented only 7.7-10.8% and 6.6-12.1% of retrieved sequences in December 2017 and May 2017 respectively. The most abundant and frequently observed classes were *Gammaproteobacteria* (21.4-31.0%), *Alphaprotobacteria* (17.4-21.0%), *Planctomycetacia* (2.8-18.3%), *Bacteroidia* (4.2-15.2%) and *Oxyphotobacteria* (4.6-10.7%).

Similarly to the NMDS community composition analysis (Figure 3.6) and phyla comparisons (Figure S2), there were generally larger changes in relative abundances of bacteria associated sequences with respect to sampling time points than between proximity treatments at the family level (Figure 3.7). For example, *Pirellulaceae* (within the Planctomycetes) affiliated sequences were retrieved at consistently higher relative abundances from February samples (17.6-21.1%) compared to May (7.1-9.7%) (Figure 3.7). In December samples however, there were higher abundances of *Pirellulaceae* affiliated sequences in direct contact samples (14.2%) compared to systemic (6.1%) and isolation (2.9%) samples. *Cyanobiaceae* affiliated sequences were consistently higher in February (10.0-14.7%) compared to December (4.1-6.3%) and May (2.1-6.4%), while, by contrast, *Flavobacteriaceae* (*Bacteroidetes*) and *Nitrosopumilaceae* (*Proteobacteria*) displayed the opposite trend, having lowest relative abundance of retrieved sequences in February (2.6-5.7% and 0.9-1.8% respectively) compared to December (7.1-9.2% and 2.9-7.5%) and May (7.1-9.5%; and 5.8-14.4 %). For all these families no clear effect of proximity treatment (direct contact, systemic or isolation) was observed (Figure 3.7). Interestingly, *Endozoicomonadaceae* affiliated sequences represented only 1.5% of retrieved sequences within the direct contact samples compared to 9.3% for isolation samples at the May sampling point. During February sampling, at the peak of *Sargassum* biomass (Figure 3.3a), the relative abundance of *Endozoicomonadaceae* affiliated sequences was much lower,

representing only 0.15% of sequences retrieved from systemic treatment samples, and this was not observed in any other February proximity treatment (Figure 3.7).

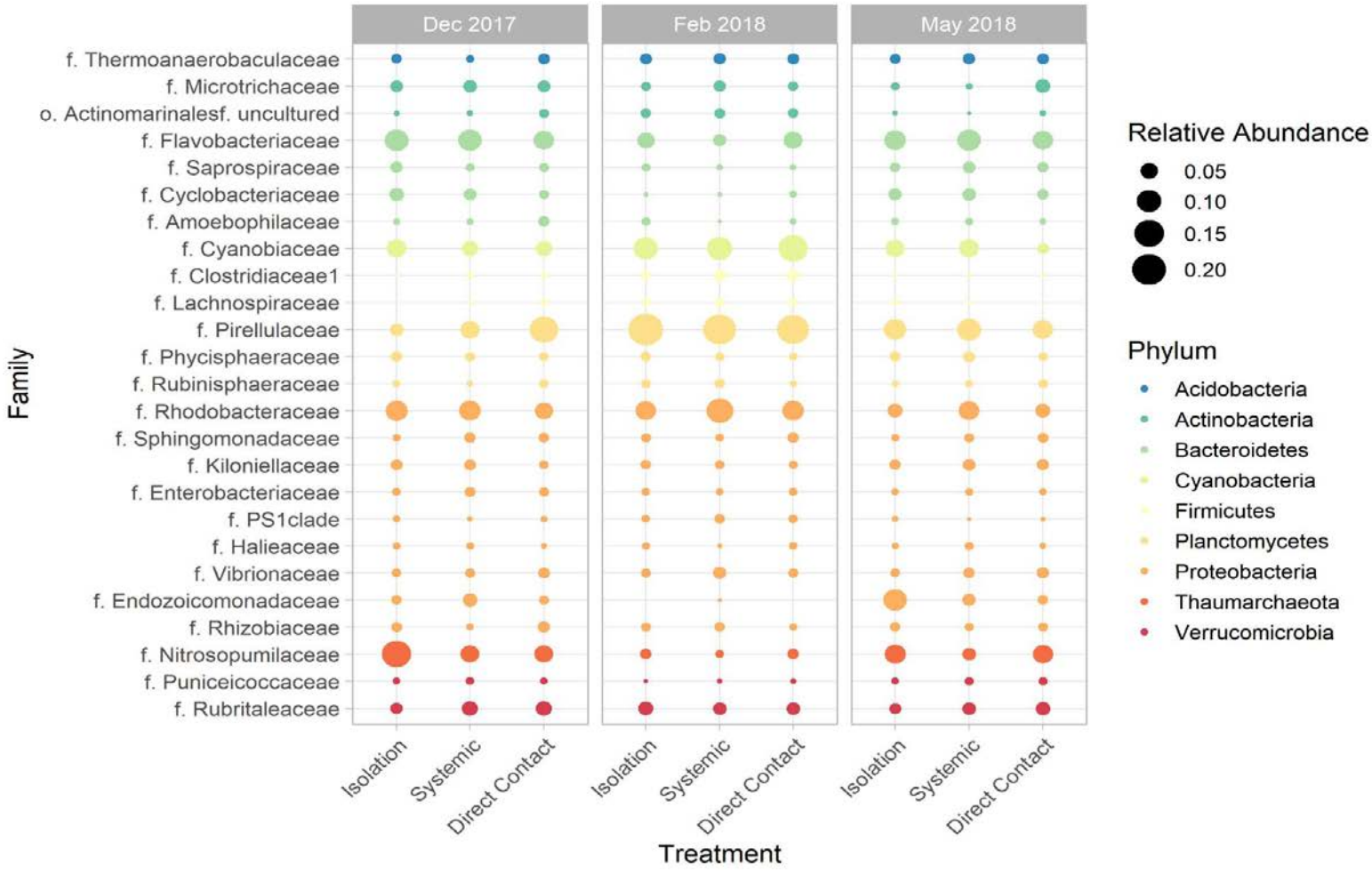


Figure 3.7 Top 25 most abundant bacterial families associated with all samples and replicates of *M. aequituberculata* across sampling time points and proximity treatment. Families grouped by associated phylum (colour).

3.4.2.2 *Sargassum* species bacterial community analysis

Diversity comparisons

Diversity analysis of the *Sargassum* species bacterial communities revealed there was a significant interaction between species and time. The richness and diversity of the *Sargassum* bacterial community was significantly lower in December compared to February and May (Faith's richness: ANOVA, $p < 0.001$; Shannon's diversity: ANOVA, $p < 0.001$) (Figure 3.8a, b). No significant differences in the richness and diversity of the *Sargassum* bacterial community was observed across proximity treatment (Faith's richness: ANOVA, $p = 0.78$; Shannon's diversity: ANOVA, $p = 0.60$) (Figure 3.8c, d).

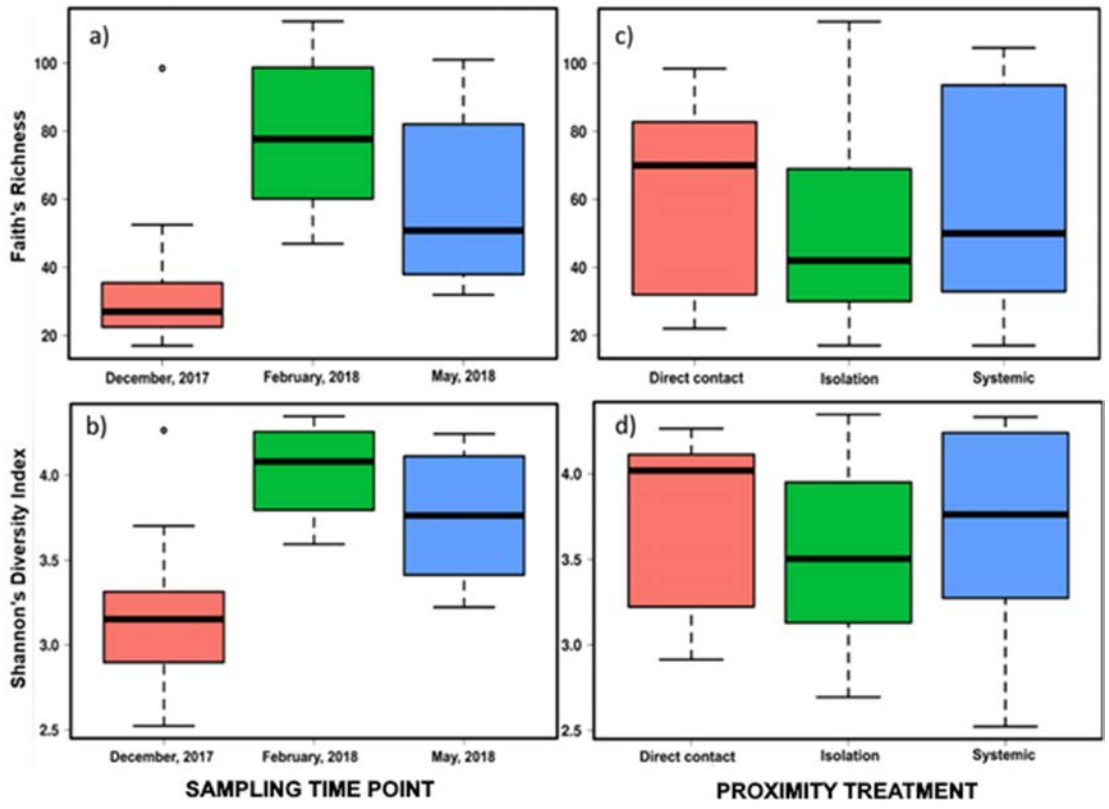


Figure 3.8 Alpha diversity of *Sargassum* species across sampling month and proximity treatment. Faith's richness (a) and Shannon's diversity (b) observed to be significantly lower in December compared to February and May sampling time points. No significant difference in the *Sargassum* species Faith's richness (c) and Shannon's diversity (d) across proximity treatment.

Bacterial community composition

Bacterial community composition analysis showed that there was a significant interaction between species and time. The *Sargassum* species bacterial community composition was significantly different between December and May (PERMANOVA: $R^2 = 0.116$, $p = 0.013$) (Figure 9a). Again, however, there was no significant difference in the bacterial community composition of *Sargassum* species across the proximity treatments direct contact, isolation and systemic (PERMANOVA: $R^2 = 0.601$, $p = 0.13$) (Figure 3.9b).

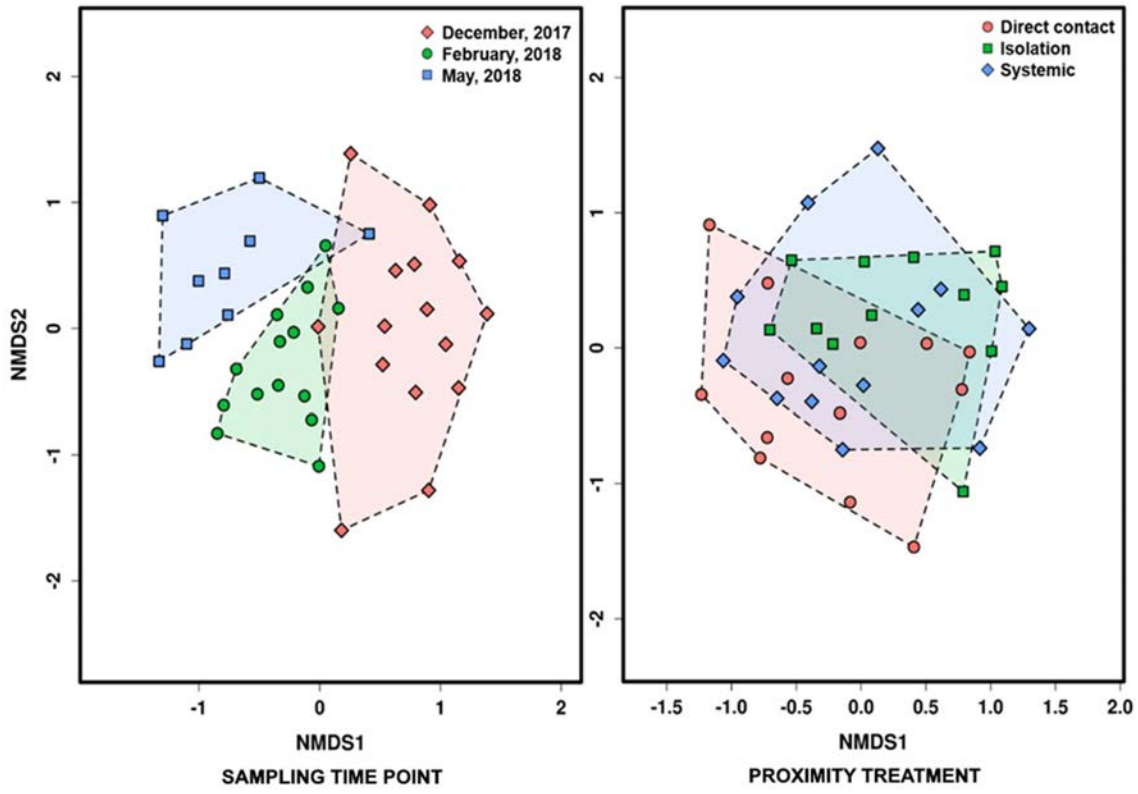


Figure 3.9 Bacteria community composition analysis of *Sargassum* species. a) December community composition of *Sargassum* species observed to be significantly different from the May sampling time point (December, red; February, green; May, blue). b) No changes in the *Sargassum* species microbial community observed across proximity treatments.

Sequences associated with 40 bacterial Phyla and 4 archaeal Phyla were identified within the 16S rRNA dataset across *Sargassum* species samples. Archaeal phyla, as in the coral samples, represented less than 2% of all retrieved sequences. Bacteria affiliated with the *Proteobacteria*

phyla were the most abundant within the *Sargassum* species bacterial community (39.2-53.3.0%) (Figure S1, 2). As observed with the *M. aequituberculata* samples, there were no major shifts in the bacterial community profiles of *Sargassum* with respect to proximity treatment. Across the temporal time points, the dominant phylum *Proteobacteria* was relatively consistent for the December (47.3-53.3%), February (44.7-47.0%) and May (39.2-47.0%) sampling points (Figure 3.10). Similarly the second and third most abundant Phyla, *Bacteroidetes* (18.1-26.8%) and *Cyanobacteria* (8.2-14.6%), remained relatively consistent for all temporal and proximity samples. The most abundant and frequently observed classes were *Bacteroidia* (18.7-27.0%) *Gammaproteobacteria* (16.4-28.0%) and *Alphaprotobacteria* (20.1%).

At the family level, there were generally larger changes in relative abundance of sequences derived from the *Sargassum* species with respect to sampling time points than between proximity treatments (Figure 3.10). For example, *Rhodobacteraceae* (Proteobacteria) affiliated sequences were consistently the most abundant family retrieved across all *Sargassum* samples, and most abundant in December samples (14.3-21.2%) compared to those of February (10.3-12.9%) and May (9.3-16.2%). Their relative abundance, however, was similar for the proximity treatments of direct contact, isolation and systemic samples at each of these sampling time points (Figure 3.10). Within the Bacteroidetes, *Saprospiraceae* affiliated sequences were generally in higher abundances from February (9.5-17.2%) and December samples (6.1-15.0%) compared to May (5.1-7.5%). The highest abundance of *Cyanobiaceae* (Cyanobacteria) affiliated sequences were retrieved from direct contact samples from February (4%), with no sequences recovered from the December isolation samples.

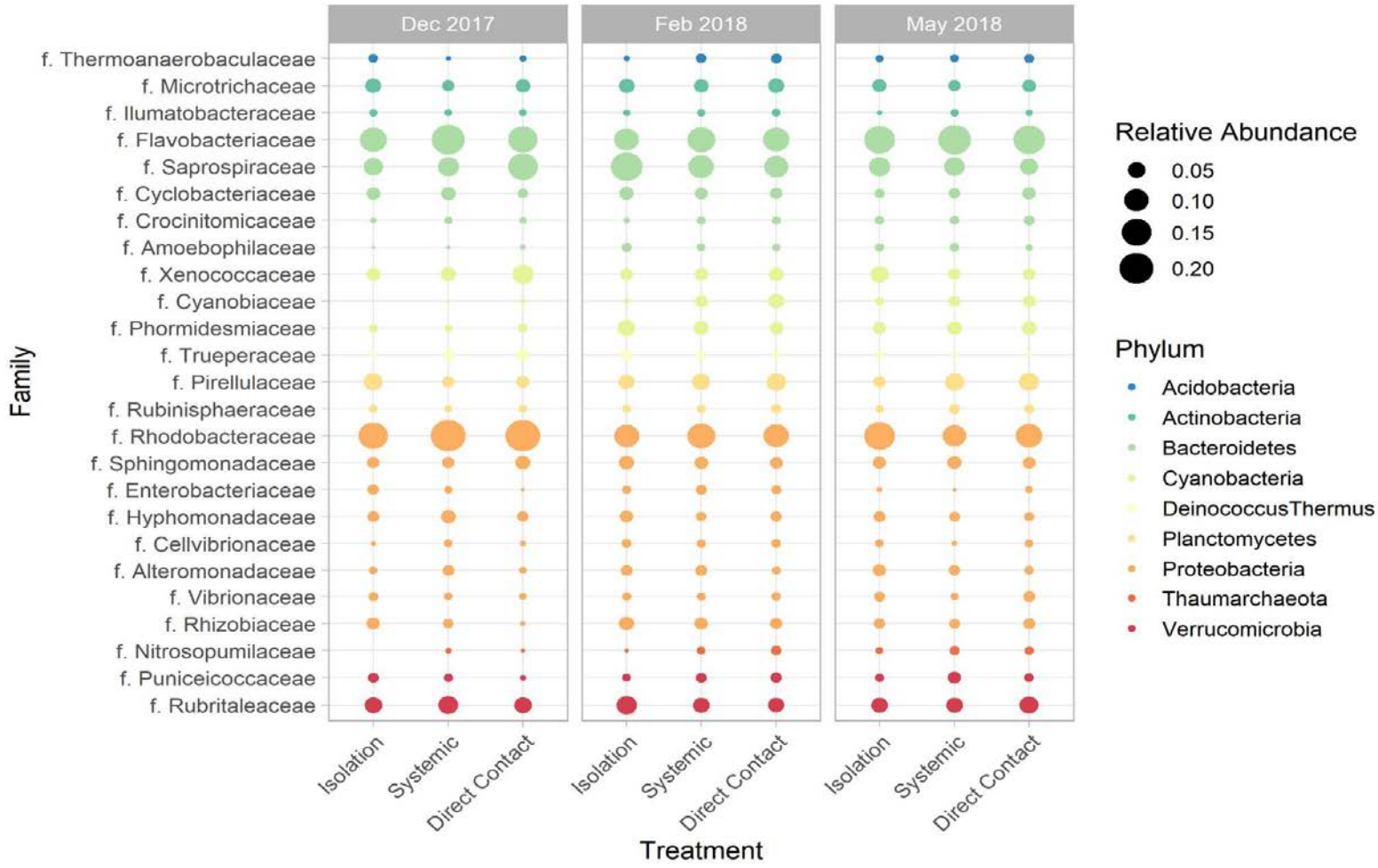


Figure 3.10 Top 25 most abundant bacterial families associated with all samples and replicates of *Sargassum* species across sampling time points and proximity treatment. Families grouped by associated phylum (colour).

3.4.2.3 Seawater bacterial community analysis

Diversity comparisons

Seawater bacterial community diversity varied significantly over time. The richness of the seawater bacterial communities was significantly higher in February and May compared to December (Faith's richness: ANOVA, $p = 0.046$) (Figure 3.11a), and the diversity of the seawater bacterial community was higher in May compared to February and December (Shannon's diversity: ANOVA, $p = 0.05$) (Figure 3.11b).

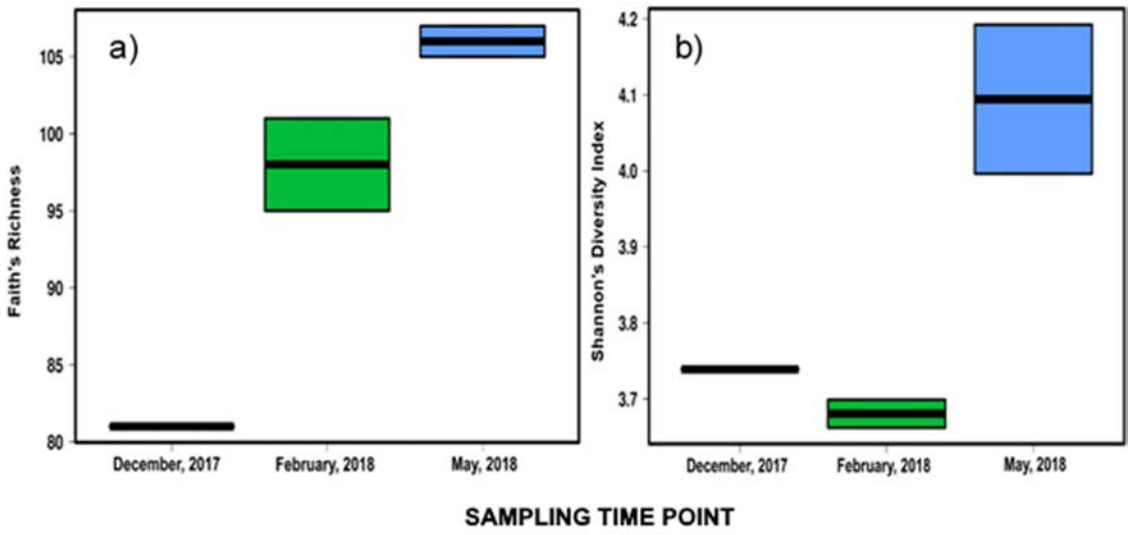


Figure 3.11 Alpha diversity of the seawater bacterial community across sampling month. a) Faith's richness observed to be significantly lower in December compared to February and May. b) Shannon's diversity observed to be significantly higher in May compared to December and February.

Bacterial community composition

At each time point, the seawater, *M. aequituberculata* and *Sargassum* species bacteria community composition differed significantly (DECEMBER – PERMANOVA: $R^2 = 0.191$, $p < 0.001$; FEBRUARY – PERMANOVA: $R^2 = 0.256$, $p < 0.001$; MAY – PERMANOVA: $R^2 = 0.234$, $p = 0.003$) (Figure 3.12a, b, c).

Sequences associated with 25 bacterial Phyla and 3 archaeal Phyla were identified within the 16S rRNA dataset across seawater samples. Similar to the *Sargassum* and *M. aequituberculata* bacterial community datasets, bacteria affiliated with *Proteobacteria* represented the highest abundance of retrieved sequences across all time periods, but were most abundant in May (47.1%) compared to December (36.9%) and February (37.2%) (Figure S2). By contrast, the second most abundant Phyla, *Cyanobacteria* (overall: 33.3%) were retrieved in lowest relative abundances in May (27.5%) compared to February (37.8%) and December (36.3%), while *Bacteroidetes* were consistently retrieved across all sampling time points (December: 14.2%; February: 12.7%; May: 12.1%). The most abundant and frequently observed classes were *Oxyphotobacteria* (33.9%), *Alphaprotobacteria* (24.7%), *Gammaproteobacteria* (13.3%), and *Bacteroidia* (12.5%). Within the Cyanobacteria, *Cyanobiaceae* affiliated sequences were in highest relative abundance in December (45.0%) and February (45.4%) samples but represented only 33.0% of sequences in May. The next most abundant family, *Flavobacteriaceae* (Bacteroidetes), was in much lower abundances, and was retrieved consistently from all sampling periods (December: 8.8%; February: 8.8%; May: 9.4%) (Figure 3.13).

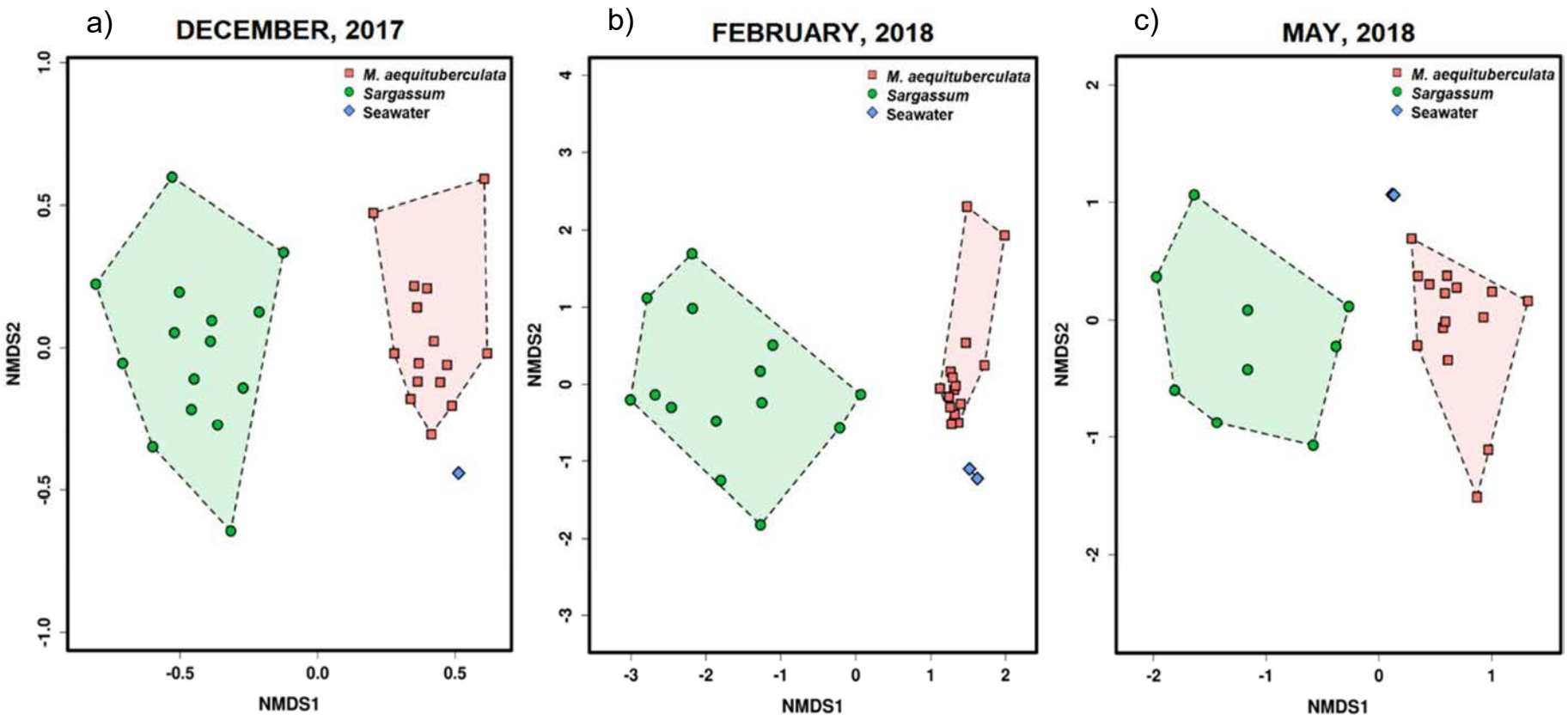


Figure 3.12 Bacteria community composition analysis of seawater (blue) compared to *Sargassum* species (green) and *M. aequituberculata* (red) over time. Over all sampling time points, the *Sargassum* species and *M. aequituberculata* bacterial communities remain distinct from each other and seawater.

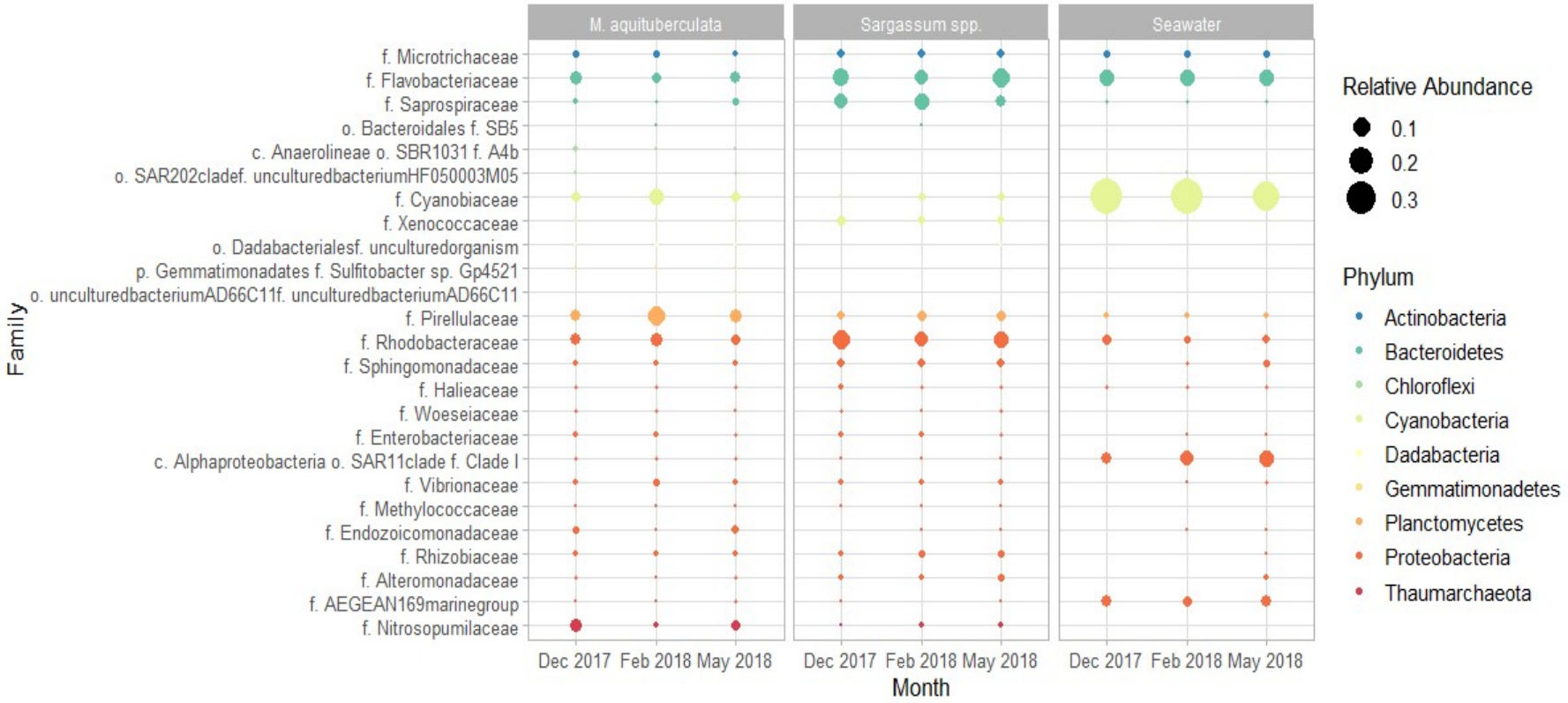


Figure 3.13 Top 25 most abundant bacterial families associated with all samples and replicates of *Sargassum*, *M. aequituberculata* and seawater across sampling time points to compare bacterial community compositions. Families grouped by associated phylum (colour)

3.5 Discussion

3.5.1 Temporal patterns in the benthic assemblage of Geoffrey Bay

Increased frequency of abiotic stressors has the potential to shift some tropical reefs from coral to macroalgae dominance (Dubinsky and Stambler 2011). Colonisation of space by macroalgae previously occupied by coral can be interpreted as competition between coral and macroalgae (Box and Mumby 2007, Cheal et al. 2010). In particular, on some inshore reefs of the GBR, *Sargassum* species overgrow coral in summer months, outcompeting coral for space, light and nutrients (Martin-Smith 1992, Schaffelke and Klumpp 1997, Ceccarelli et al. 2018). Even when *Sargassum* biomass senesces in winter months, lower stipes and holdfasts of the algae remain across coral reefs (Loffler et al. 2018), continuing to take up space and compete with coral.

In this chapter, *Sargassum* density at Magnetic Island was recorded over time and demonstrated seasonal differences with highest density (as indicated by canopy height and percent cover) observed in the summer months (December and February), and with a significant decline in density at the end of autumn (May) (Figure 3). The percent cover of *Sargassum* recorded was consistent with recent assessments by Ceccarelli et al. (2019), who observed ~30-40% cover of *Sargassum* at Magnetic Island. Tropical variants of the *Sargassum* genus show pronounced seasonality with respect to growth, reproduction and senescence (Critchley et al. 1991, Kaehler and Williams 1996, Schaffelke and Klumpp 1997). The increase in growth and reproduction of *Sargassum* species has been observed previously at Magnetic Island as occurring from late spring to peak density in mid-late summer, with lower density from autumn to winter (Martin-Smith 1993). The growth and senescence cycles of tropical *Sargassum* species are linked to changes in seawater temperature. Fulton et al. (2014) observed peak *Sargassum* canopy biomass on Ningaloo Reef (Western Australia), with warmer summer temperatures and larger biomass in 2011, and cooler summer temperatures and smaller biomass in 2012.

Macro-scale impacts of contact between macroalgae and coral have been well documented (i.e. coral tissue paling and necrosis, and localised bleaching (Diaz-Pulido et al. 2009, Morrow et al. 2011, Barott and Rohwer 2012)). Changes within host microbiomes as a result of coral-algal contact, however, are often overlooked. The impacts associated with direct coral-algal contact at the micro-scale are likely to be greatest at these peak summer *Sargassum* densities (Figure 3), when the chance of direct contact between coral and *Sargassum* is highest.

3.5.2 Temporal changes in the *Sargassum* species, *M. aequituberculata* and seawater bacteria communities

The bacterial communities of *Sargassum*, *M. aequituberculata* and seawater were all significantly different from each other, and all displayed changes across sampling time points. The largest temporal shifts were observed within the seawater bacterial communities, with the *Cyanobiaceae* representing nearly half of the retrieved sequences at the summer sampling time points (December and February), though only approximately a third of all retrieved sequences for the May sampling point. *Cyanobiaceae* are autotrophic microorganisms (for example, ASV *Synechococcus* observed throughout the dataset), commonly associated with other photosynthetic organisms (Charpy et al. 2012, Cornet et al. 2018). Thus, it is likely that *Cyanobiaceae* bacteria are more abundant throughout summer periods due to greater access to light and warmth for increased metabolic processes (Frade et al., *in press*). Recent research has highlighted seawater microbiomes are highly responsive to changes within environmental conditions such as increased seawater temperatures, and these responses show a uniform response pattern over time (Glasl et al. 2019). Future research investigating temporal changes in the seawater microbiome would therefore benefit from repeated long term monitoring to examine the consistency of these community shifts.

Bacteria belonging to the family *Endozoicomonadaceae* also displayed a seasonal response. In *M. aequituberculata* samples, *Endozoicomonadaceae* affiliated sequences were in highest

abundance in May (1.5-9.3%), but were much lower in February (0-0.15%) (Figure 7) when the density of *Sargassum* species was highest. Bacteria belonging to the family *Endozoicomonadaceae* are associated with many coral genera and other marine invertebrates (Bayer et al. 2013, Neave et al. 2016, Pogoreutz et al. 2018). Genomic sequencing of *Endozoicomonadaceae* bacteria has highlighted the potential for important functional roles, such as transport of organic molecules and the synthesis of amino acids (Neave et al. 2017), contributing positively to coral health (Shiu and Tang 2019). Various studies have shown a reduction in the relative abundance of bacteria belonging to the *Endozoicomonadaceae* family in the microbiome of corals under environmental stress (e.g. bleaching, increased ocean acidification and disease) (Glasl et al. 2016, O'Brien et al. 2018, Pogoreutz et al. 2018, Pollock et al. 2019). A reduction in this bacterial family in response to environmental stressors may compromise coral health. To explore how declines in bacteria associated with *Endozoicomonadaceae* may influence overall coral host health during periods of high *Sargassum* density*,* future work should incorporate metagenomics to identify the genomic metabolic potential of coral microbial communities, along with their gene expression responses through metatranscriptomics. This could then be linked to host transcriptomics to correlate *Endozoicomonadaceae* functional response with host metabolic changes, and represent a more complete understanding of interactions between the coral microbiome and host during periods of high *Sargassum* abundance and increase coral-algal interactions.

Lower relative abundances of *Saprospiraceae* affiliated sequences were observed in the *Sargassum* microbial community in May compared to sampling in December. *Saprospiraceae* has previously been linked to breakdown of complex molecules and waste material (McIlroy and Nielsen 2014), carbon cycling (Khan et al. 2007, Lee 2007, Oh et al. 2009) and localised from macroalgal biofilms and macroalgae microbial communities (Burke et al. 2011, Miranda et al. 2013). The lower relative abundance of *Saprospiraceae* affiliated sequences is consistent

with potentially reduced nutrient cycling requirements of *Sargassum* species when entering the senescence cycle (peak biomass in summer followed by decline in autumn). Further host and microbial transcriptomic approaches are required, however, to establish if changes in *Saprospiraceae* communities are linked to *Sargassum* metabolic changes throughout this senescence cycle.

Temporal changes in the *Sargassum* density observed throughout this study have highlighted scope for future research to investigate links between increased *Sargassum* abundance and indirect coral-algal interaction models. Increased macroalgae abundance has been linked to the DDAM model and microbialisation of coral reefs (Barott and Rohwer 2012, Haas et al. 2016). Increases in macroalgae abundance releases DOC into the environment, causing increases in heterotrophic microbes that outcompete coral for nutrients (Roach et al. 2017). These processes are predicted to contribute to continued degradation of corals throughout periods of high macroalgae abundance (Barott and Rohwer 2012, McDole Somera et al. 2016). Moreover, recent research has highlighted the potential for the seawater microbiome to be used as an indicator of environmental change (Glasl et al. 2019). As the seawater microbiome is amenable to abiotic fluctuations, assessing how abundant macroalgae may influence the seawater microbial community could allow for an empirical assessment of the DDAM model and microbialisation, and their effects upon coral health.

3.5.3 Direct contact interactions observed in the *Sargassum* species and *M. aequituberculata* bacterial communities

The microbial communities associated with both *Sargassum* and *M. aequituberculata* appeared to be stable despite direct contact between these species. One interesting observation, however, was the shift in the *Endozoicomonadaceae* related sequences associated with *M. aequituberculata* samples. Thought not found to be statistically different between proximity treatment, *M. aequituberculata* displayed lower average relative abundance of

Endozoicomonadaceae bacteria for direct contact samples compared to isolation samples. As previously highlighted, *Endozoicomonadaceae* associated bacteria are prevalent across multiple coral species (Neave et al. 2016, Shiu and Tang 2019), and have a putative beneficial role in the coral holobiont (Neave et al. 2017, Shiu and Tang 2019), with reductions in their relative abundance observed for stressed coral microbiomes (Glasl et al. 2016, O'Brien et al. 2018, Pogoreutz et al. 2018, Pollock et al. 2019). Lower relative abundances of sequences associated with *Endozoicomonadaceae* suggests direct contact with *Sargassum* species influences the corals microbiome and potentially overall health.

Bacteria associated with *Cyanobiaceae* (*Oxyphotobacteria*) were consistently abundant within *M. aequituberculata* across all proximity treatments (~5-10%). For *Sargassum*, however, the relative abundance of *Cyanobiaceae* affiliated sequences was lower overall (1-2%), and particularly so in isolated *Sargassum* samples (Figure 7). The comparatively higher relative abundance of *Cyanobiaceae* sequences retrieved from *M. aequituberculata* compared to *Sargassum* may be explained by the observation that the coral mucus bacterial communities can be influenced by the surrounding seawater microbial community (Frade et al. 2016). DNA derived from all coral samples included coral mucus and coral tissue. *Cyanobiaceae* were the most abundant taxa in the seawater (Figure 13), and these sequences could be derived from that boundary layer of seawater, mucus and coral tissues. The coral mucus is the first point of direct contact with macroalgae, which may also explain why the relative abundance of *Cyanobiaceae* affiliated bacterial sequences was higher in *Sargassum* direct contact samples.

Previous research into microbial coral-algal interactions has provided evidence for allelopathy (Morrow et al. 2012, Ritson-Williams et al. 2016), stimulation of disease (Barott and Rohwer 2012), and dysbiosis of the coral bacteria community (Pratte et al. 2018). For example, allelochemicals produced by the brown macroalgae *Lobophora* genus have been active against

coral larvae settlement, and are associated with a loss of *Endozoicomonas* bacteria within coral tissues (Morrow et al. 2017). Whilst some changes in the bacterial communities of *M. aequituberculata* and *Sargassum* species were observed in direct contact samples, overall there was little effect of proximity between the two organisms. Furthermore, there were no signs of disease, tissue necrosis or lesions on *M. aequituberculata* in direct contact with *Sargassum* species throughout field surveys (Table S2), these being common responses to allelopathic action (Rasher and Hay 2010, Greff et al. 2017). There is debate as to whether *Sargassum* is an allelopathic alga (Rasher and Hay 2014), and in particular *Sargassum polycystum*, a common species at Magnetic Island (Martin-Smith 1993), has been observed as having no allelopathic effect on corals (Bonaldo and Hay 2014). Thus, it is likely that direct contact between *Sargassum* species and *M. aequituberculata* is not a source of allelopathic action, and this may partially explain why no extensive microbial direct interactions were observed.

3.5.4 Stability of the *M. aequituberculata* bacteria community with proximity to *Sargassum* species at Magnetic Island

Overall, the *M. aequituberculata* bacteria community remained consistent and distinct from *Sargassum* species and seawater. *Pirellulaceae* affiliated sequences were observed in consistently high relative abundances in *M. aequituberculata* samples, with no discernable response to proximity treatment (Figure 7). Bacteria belonging to the family *Pirellulaceae* have been frequently retrieved from coral microbiome studies (Lawler et al. 2016, Weiler et al. 2018, Kellogg 2019) and attributed with nitrogen cycling (Gade et al. 2004, Mohamed et al. 2010). Furthermore, *Gammaproteobacteria* related sequences were in consistently high relative abundances with respect to both proximity treatments and sampling time point. Bacteria belonging to the class *Gammaproteobacteria* have previously been associated with carbon, nitrogen and sulphur cycling within the coral microbiome (Raina et al. 2009, 2016, Bourne et

al. 2013), and, like *Pirellulaceae*, have a potentially important role in maintaining host health of *M. aequituberculata.*

The *M. aequituberculata* bacterial community from Magnetic Island was found to be relatively unchanging with respect to direct algal interactions (Figures 6, 7). This is consistent with previous studies that have shown the microbial stability of the *Montipora* species throughout periods of environmental stress. Gonzalez-Pech at al. (2017) identified *Montipora digitata* transcriptomic resilience in response to change in pH, for example, and ascertained that the microbial community had the capability to acclimatise to extended periods of low pH. Furthermore, Van De Water et al. (2017) showed *M. aequituberculata* is capable of maintaining a stable bacterial community under elevated seawater temperatures by regulation of genes involved in stress and immune response processes. Thus, it is likely that species of coral belonging to the genus *Montipora* may be host to an innate microbial stability throughout periods of environmental stress and change, and results from this study complement previous observations such as those cited above. This microbiome stability has been consistently observed for adult corals, although increased macroalgae abundance has been shown to inhibit juvenile coral growth and settlement by competing for space (Norström et al. 2009, Ceccarelli et al. 2018), and in some cases negative impacts from coral-algal interactions have been limited to affecting early life stages of coral (Kuffner et al. 2006, Leong et al. 2018, O'Brien and Scheibling 2018). Thus, future research should explore microbial effects of contact between *Sargassum* and coral larvae and planulae, to investigate how direct contact and high *Sargassum* abundance can impact coral recruitment.

One explanation as to why the bacterial community of *M. aequituberculata* remained consistent in response to direct contact with *Sargassum* species in this study may be historical ecological factors at Magnetic Island where the samples were collected. Macroalgae abundance has been

steadily increasing since the 1980s (Ceccarelli et al. 2018) linked to increased nutrient input from the Townsville port and coastal development (Bak 1978, Browne et al. 2013). Whilst abundance of coral across the fringing reefs of Magnetic Island has significantly declined with the increase of macroalgae, corals that remain display resilience to the challenging effects of their environment, including poor water quality, high sedimentation and high macroalgae abundance. Magnetic Island's fringing reefs still host a diverse range of corals including *Acropora*, *Montipora*, *Porites* and *Dipsastrea* (previously *Favia*) (Marshall and Baird 2000, Bourne 2005, Glasl et al. 2019, Saha et al. 2019), and have displayed resilience in response to bleaching and cyclone events (Ayling and Ayling 2005). At Magnetic Island, *Montipora* species and *Sargassum* species have successfully grown alongside each other as *Sargassum* species' abundance has gradually increased over time, with *Montipora* species as one of the dominant coral present on reefs at Magnetic Island (AIMS, 2019). Thus, as a result of ongoing interactions with macroalgae, *M. aequituberculata* and its associated bacterial community have developed resilience to periods of high algal abundance. To test this hypothesis, it is important to characterise host responses and microbiome patterns of *M. aequituberculata* from different locations across the GBR where macroalgae abundance is lower and there is greater separation between macroalgae and coral colonies. If combined with metagenomic and transcriptomic analyses, this could be expanded to characterise the stability of other, potentially more susceptible coral taxa at various levels of macroalgae abundance.

3.6 Conclusions

The objective of this Chapter was to investigate the effect of proximity on the microbial communities of *Sargassum* species and *M. aequituberculata*. Small shifts in the average relative abundance of the *M. aequituberculata* and *Sargassum* microbial communities direct contact samples were observed, which may be linked to points where the samples were physically touching. Overall, however, proximity did not have a discernable effect on either

the *M. aequituberculata* or *Sargassum* bacterial communities. Importantly, this result suggests the *M. aequituberculata* and its associated bacterial community may be resilient to contact with *Sargassum* potentially due to historically high abundances of the macroalgae at Magnetic Island. Both the *Sargassum* species and *M. aequituberculata* were found to be distinct from the seawater microbial community and changed across sampling time points. The observed changes in the *Sargassum* and *M. aequituberculata* samples over time may be linked to changes in the *Sargassum* species density from December 2017/February 2018 to May 2018. Future metagenomic research should be used to investigate the genomic metabolic potential of the *M. aequituberculata* and *Sargassum* microbial communities, along with their gene expression response to changes in *Sargassum* density through metatranscriptomics. This could be linked with host transcriptomics to correlate the microbial functional response (particularly *Endozoicomonadaceae* and *Saprospiraceae*) with host gene expression change. Whilst few substantive microbial coral-algal interactions were observed, the information collected provides a species specific dataset exploring the *Sargassum* and *M. aequituberculata* bacterial communities over time and with proximity. The methods used to determine the effect of direct contact between *Sargassum* and *M. aequituberculata* can also be applied to other coral and algal species to explore whether proximity between coral and macroalgae negatively influences host bacterial communities, with the potential to reduce coral health on the inshore GBR.

4 General Discussion

4.1 Characterising the macroalgae microbiome

Degraded reef systems can be susceptible to shifts from coral to macroalgae-dominated environments (McCook et al. 2001, Mumby 2009, Ceccarelli et al. 2018). On inshore regions of the GBR, coastal development, increased sedimentation and higher nutrient inputs are degrading reefs, with some experiencing high macroalgal abundance (De'ath et al. 2010, Ceccarelli et al. 2018, 2019). Coral health is dependent on maintenance of associated microbial partners which include photosynthetic dinoflagellates (*Symbiodiniaceae*) and a range of other bacteria (Rohwer et al. 2002, Ainsworth et al. 2010). However, environmental stress can disrupt the stability of these microbial communities, subsequently impacting coral health (Bourne et al. 2008, 2009, Meron et al. 2011, Glasl et al. 2016). As macroalgae dominance increases on some reefs, it is important to assess if macroalgae presence and direct contact with coral affects coral microbiomes.

To investigate the potential negative effects macroalgae may have on coral microbiomes, it is important to first provide a baseline assessment of the microbial communities associated with macroalgae. In the Caribbean, Barott et al. (2012) revealed different functional groups of macroalgae have varied microbial communities. In temperate Australia, Burke et al. (2011b) defined a species-specific core microbial community of *Ulva australis*. Current attempts to define macroalgae microbial communities on the GBR have, however, been limited. In this thesis, Chapter 2 explored the microbial community of two *Sargassum* species (*S. aquifolium, S. polycystum*) to begin to define macroalgae microbiomes on the GBR. The study took place

at Magnetic Island, an inshore reef of the central GBR with a historical high abundance of *Sargassum* (Mapstone et al. 1992, Martin-Smith 1993, Ceccarelli et al. 2019).

Chapter 2 revealed that the diversity and bacterial community composition of two macroalgae species, *Sargassum aquifolium* and *Sargassum polycystum*, were similar. The samples were collected from the same location and time point, with previous studies also showing that macroalgae associated microbial communities are similar when sampled from the same environment (Campbell et al. 2015, Perkins et al. 2016, Glasl et al. 2019). As coral reef health on the GBR is threatened by spatially variable environmental and anthropogenic stressors, it is pertinent therefore, to replicate Chapter 2 on *Sargassum* species from different locations and time points on the GBR. This will aid understanding the degree to which the *Sargassum* bacterial community is species-specific or dependent on the surrounding environment, and highlight how changing environmental conditions may influence the *Sargassum* microbiome.

Importantly, results from Chapter 2 showed microbial community differentiation between *Sargassum* regions (holdfast, basal growth, stem (primary axis), leaf and biofilm). Many studies investigating the bacterial community of macroalgae have focused solely on the biofilm of the alga (Burke et al. 2011, Barott and Rohwer 2012, Egan et al. 2013, Glasl et al. 2019), however findings from Chapter 2 suggests it is important to also include other tissue regions to characterise the complete bacterial community of macroalgae. From the findings of Chapter 2, the biofilm was also identified as the most useful region for investigating direct coral-algal interactions due to be being highly diverse and the first point of contact between the alga and coral.

The next steps, following elucidation of the baseline microbial communities of *Sargassum* species, is to assess the functional roles of the bacteria within the holobiont and their contribution to host health. In terrestrial plants, bacterial and fungal microbial communities

contribute to sustaining host health (Zhang and Yao 2015, Wallace et al. 2018). Bacteria associated with the plant *Arabidopsis thaliana*, for example, have been shown to increase root growth (Berg 2009). Like plants, algae produce nutrient sources through photosynthesis (Muscatine et al. 1969, Lloyd et al. 1977) and are often considered 'plants of the sea'. While macroalgae are less complex organisms than plants, which lack vascular systems and can sometimes be unicellular (Bhattacharya and Medlin 1998), it is likely microbes may play a similar role in sustaining algae health as in plants. The functional role of bacterial sequences retrieved from Chapter 2 can be speculated, for example, *Sargassum* leaf and biofilm communities were dominated by bacteria associated with nutrient cycling (for example *Loktanella* and *Saprospiraceae*), and the basal growth and holdfast were dominated by bacteria associated with anaerobic respiration (*Firmicutes* and *Geobacter*). Within terrestrial plants, bacteria associated with *Geobacter* has been sequenced from the roots of rice plants in paddy fields, and directly associated with aiding anaerobic respiration, highlighting an ability to survive in anoxic environments (Ikenaga et al. 2003). Additionally, *Chlorophyta* (green) algae and terrestrial plants are thought to host similar Plant Growth Promoting Bacteria (PGPB), primarily belonging to the bacterial phyla *Proteobacteria* and *Bacteroidetes* (Ramanan et al. 2016). Throughout Chapter 2, bacteria belonging to both *Proteobacteria* and *Bacteroidetes* were highly abundant within the *Sargassum* species bacterial community. Hence further investigation should explore if *Sargassum* species are also host to PGPB which may aid in supporting healthy growth pathways within the alga. Using 16S rRNA gene data alone in characterising microbe-algae function is, however, problematic. Thus, it is important to examine the function of different regions of the macroalgae. Metagenomics can be used to identify the genomic metabolic potential of macroalgae microbial communities, along with their gene expression responses through metatranscriptomics. This could then be linked to host transcriptomics and proteomics to correlate microbial functional responses with host metabolic

changes, and represent a more complete understanding of interactions between the macroalgae microbiome and host.

Microscopy proved a useful tool to explore macroalgae associated bacterial communities. Fluorescent *in situ* hybridisation revealed various structures and clusters of potential bacteria. Further research should use species specific probes to target potentially important bacteria for *Sargassum* species, specifically *Saprospiraceae*, to validate 16S rRNA gene amplicon observations. Using microscopy has highlighted potential fungal morphologies in Chapter 2. Fungal communities are essential to terrestrial plant microbiomes, for example *Trichoderma* species can supply macro- and micro-nutrients to the plant host primarily through nitrogen cycling (Contreras-Cornejo et al. 2009). Fungal communities have also been associated with Antarctic macroalgae (Loque et al. 2010), and algae represent the second largest source of marine fungi (Berg 2009). Fungal communities in Antarctic macroalgae may be linked to carrageenolytic and agarolytic activites that breakdown polysaccharides, particularly carrageen and agar found within macroalgae (Furbino et al. 2018, Ogaki et al. 2019). Such fungi are thought to breakdown macroalgae biomass and release nutrients into the surrounding environment (Furbino et al. 2018). Future research should attempt to characterise fungal communities in marine environments and their potential functional roles in tropical macroalgae species. Whilst this is an important step in defining the entire macroalgae microbiome, characterising fungal communities (mycobiomes) in marine environments is currently limited. ITS primers typically used for fungal amplification also amplify other eukaryotes (e.g. Symbiodiniaceae) (Scholz et al. 2016, Hume et al. 2018) resulting in limited representation of fungal communities (Amend et al. 2019). To accurately characterise marine mycobiomes standardization of fungal primers and sampling techniques need to be established.

4.2 Direct microbial coral-algal interactions on the inshore GBR, Magnetic Island

A comprehensive understanding of the micro- and macro-scale effects of coral-algal interactions on coral health is required to predict reef ecosystem states under macroalgae-dominated regimes. Previous studies have identified that negative effects on coral health occurs through direct contact with algae resulting in abrasion of coral tissues, inducing coral bleaching and necrosis (Rasher and Hay 2010, Vieira et al. 2016a). Some indirect microbial processes may also have negative implications for coral health. For example, the DDAM model and the microbialisation index postulate that indirectly high macroalgal abundance can impact coral health through releasing excess DOC, triggering a switch from autotrophic to heterotrophic microbes thought to deprive coral of the necessary nutrients they need for survival, leading to a coral degradation (Barott and Rohwer 2012, Haas et al. 2016, McDole Somera et al. 2016). Chapter 3 focused on direct contact interactions between the host bacterial communities of macroalgae and coral, assessing if direct contact influences the bacterial communities of coral and algae, and infer if this may impact host health using the microbiome as a proxy for health through destabilisation.

The bacterial community of *Sargassum* (macroalgae) and *M. aequituberculata* (coral) samples were collected across three proximity treatments (isolation, direct contact and systemic proximity) at Magnetic Island in the Central GBR. Samples were also collected over three time points (December 2017, February 2018, May 2018) alongside benthic community surveys to examine how the host bacterial communities and proximity interactions changed over time in relation to the senescence cycle of *Sargassum*. Overall, the *M. aequituberculata* bacteria community remained consistent across proximity treatments, which may be reflective of the tolerance of this species, and explain its ecological success despite the poor water quality

(Schaffelke et al. 2012) and historical abundance *Sargassum* on reefs at Magnetic Island (Martin-Smith 1993, Schaffelke and Klumpp 1997).

Recent research has highlighted that sections of coral tissue host coral associated microbial aggregates (CAMAs) and that the abundance of CAMAs observed increased in corals sampled from the inshore GBR (Wada et al. 2019). Microbial communities of *Sargassum* and *M. aequituberculata* from the inshore GBR were examined in Chapter 3 using the coral tissue and mucus combined. This was due to the tissue blasting technique used to remove coral tissue for DNA extraction; an inherent limitation of coral microbiome research. Coral mucus however, can respond differently to coral tissue throughout periods of environmental stress (Glasl et al. 2016), and localisation of tissue-specific CAMA communities may have different metabolic functions contributing to the coral holobiont (Wada et al. 2019). Due to the sampling of tissue and mucus together throughout Chapter 3, it is therefore not possible to discern if any of the minor changes in abundance of bacterial taxa within *M aequituberculata* and *Sargassum* were due to contact between *Sargassum* and the coral mucus or tissue. Thus, future research investigating direct coral-algal interactions should isolate coral mucus and coral tissue samples to assess which part of the corals are more microbially sensitive to direct contact with macroalgae.

Identifying the potential resilience of *M. aequituberculata* to direct microbial changes from contact with *Sargassum*, provides the opportunity explore traits which may make the coral successful at Magnetic Island. As Chapter 3 focussed on adult *M. aequituberculata* colonies, an important step forward is identifying whether microbial communities of the early life stages of the coral (such as larvae and planulae) also remain stable in direct contact with *Sargassum*. Increased macroalgae abundance can reduce coral larvae recruitment (Webster et al. 2015) and juvenile survival (Hughes et al. 2007). Algal exudates can also poison juvenile corals which

settle on turf algae (Kuffner et al. 2006, Birrell et al. 2008b, Vermeij et al. 2009a), and limit larval metamorphosis which prevents corals making it to adulthood (Baird and Morse 2004). Nonetheless, *Montipora* at Magnetic Island appear to be successful competitors, and so it is important to evaluate if this success begins with resilient microbial communities of *Montipora* larvae. Further research should monitor changes to *M. aequituberculata* larvae and planulae microbial communities to assess if they also remain stable in direct contact with *Sargassum.*

In moderate abundances, macroalgae can positively contribute to reef biodiversity, for example by protecting sessile corals from sunlight and therefore bleaching (Jompa and McCook 2003). Whilst the diversity of corals around Magnetic Island has declined with increased macroalgae (Ceccarelli et al. 2019), the corals that remain (such as *M. aequituberculata*) most likely derive some benefit from the abundance on macroalgae around the island, via shading or reducing the competition for space with other less robust coral species. Whilst no clear evidence of *M. aequituberculata* microbial community destabilisation was observed with respect to *Sargassum* proximity however, it is possible that the coral microbial community had shifted previously and those observed reflected a dysbiotic community (Zaneveld et al. 2017). Comparing results from this study to a reef with less or no historical exposure to *Sargassum* would help clarify whether the stability of the *M. aequituberculata* microbial community is inherently stable or stable with respect to local environmental and ecological conditions.

Magnetic Island has been exposed to historical degradation from increased coastal development and sedimentation (Bak 1978, Browne et al. 2013a, Ceccarelli et al. 2018). Sedimentation from dredging the Townsville Port Channel is thought to contribute to the high macroalgae abundance at Magnetic Island (Browne et al. 2013b, Ceccarelli et al. 2018). Sedimentation can have negative effects on corals, as coral energy outputs are focussed toward removing sediment through sloughing rather than growth and photosynthesis (Abdel-salam and

Porter 1988, Anthony et al. 2007). Nonetheless, *Montipora* corals have shown resilience to changes in environmental condition (Raina et al. 2009, Harpeni and David 2011), and have also been shown to retain fecundity in areas of high sedimentation. (Padilla-Gamiño et al. 2014). Further dredging around Magnetic Island is planned during port expansion capital works (Port of Townsville, 2019), which will likely increase sediment dynamics around Magnetic Island. Based on previous literature, increased sediment dynamics around Magnetic Island may result in further declines in coral abundance (Bak 1978, Fabricius et al. 2005), and increases in macroalgae (Fabricius 2005, Fabricius et al. 2005). Magnetic Island are also host to acroporids, though in much lower abundances compared to *Montipora* (AIMS 2019), which are typically sensitive to environmental change (Shinzato et al. 2011). Since macroalgae abundance around the island will be persistently high, it is essential future research also characterises the microbial stability of other more environmentally sensitive corals in direct contact with algae, to more thoroughly understand the role of microbes in direct coral-algal interactions. Furthermore, such research could help inform restoration practices. *Sargassum* removal restoration practices are implemented at Magnetic Island to clear space in order to aid coral recovery (Ceccarelli et al. 2018). If, however, there are corals around the island which are more sensitive to contact with *Sargassum* species, it could be prudent to target the areas around these corals first to remove *Sargassum* to reduce stress associated with coral-algal direct contact.

4.3 Conclusions

The overall objective of this thesis was to examine the effect of direct contact between coral and macroalgae on host bacterial communities on the inshore GBR. Overall, the *Sargassum* and *M. aequituberculata* bacterial community structure remained stable and consistent across all proximity treatments, yet distinct shifts were observed over time and potentially related to *Sargassum* seasonal senescence cycle. The data collected provides a species specific dataset exploring the *Sargassum* and *M. aequituberculata* bacterial communities over time and with

proximity. The methods used to determine the effect of direct contact between *Sargassum* and *M. aequituberculata* can also be applied to other study sites with less historical exposure to *Sargassum* to assess if the observed microbial stability of *M. aequituberculata* is species-specific or site-specific.

Coral reefs are facing accumulated challenges from global and local anthropogenic impacts resulting in alarming declines globally. On some inshore reefs of the GBR, poor water quality, sediments and nutrients are facilitating increases in macroalgae abundance at the expense of reef building corals. These direct and indirect interactions are complex and multidimensional with microbial scale processes critical to evaluating the outcomes of these competitive interactions. Through generating baseline information on the microbiome of *Sargassum* species and documenting response of coral and algal microbiome direct interactions, this study has attempted to shine light on these important microbial processes. Whilst this study did not observe many changes in the microbial communities of *Sargassum* and *M. aequituberulcata* in relation to direct contact, further in depth work is required to ascertain how changes in the microbiomes of macroalgae and coral are influenced by direct contact and how this may subsequently impact host health. Nonetheless, in a time where corals are threatened by various environmental and anthropogenic stressors, this research is an essential step forward to more fully understand the mechanisms by which increased macroalgae abundances contribute to coral decline.

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6 Appendix

6.1 Chapter 2 Supplementary Information

Table S 1 Recipe to make ~1.5L EDTA-DMSO solution for sample preservation

Ingredient	Recipe
93.06g disodium EDTA 400 mL Milli-Q water	Dissolve EDTA in 400 mL Milli-Q water
1M NaOH/NaOH pellets	Add NaOH until solution reaches pH 8
800 mL Milli-Q water	Add 800 mL Milli-Q and autoclave
200 mL DMSO	Add 200 mL DMSO to autoclaved solution
NaCl	Add NaCl until solution is saturated (quantity varies)

Table S 2 Hybridisation buffer recipe, adapted from Hugenholtz et al. 2001 and Wada et al. 2016, used to prepare samples for fluorescent *in situ* hybridisation

Solution	Volume
5M NaCl	360 µL
1M Tris-HCl (pH 7.2)	40 µL
100% Formamide	1000 µL
Autoclaved Milli-Q water	598 µL
10% SDS	2 µL

Table S 3 Wash buffer recipe, adapted from Hugenholtz et al. 2001 and Wada et al. 2016, used to prepare samples for fluorescent *in situ* hybridisation visualisation.

Solution	**Volume**
5M NaCl	180 µL
1M Tris-HCl (pH 7.2)	1000 µL
Autoclaved milli-Q water	48.77 mL
10% SDS	50 µL

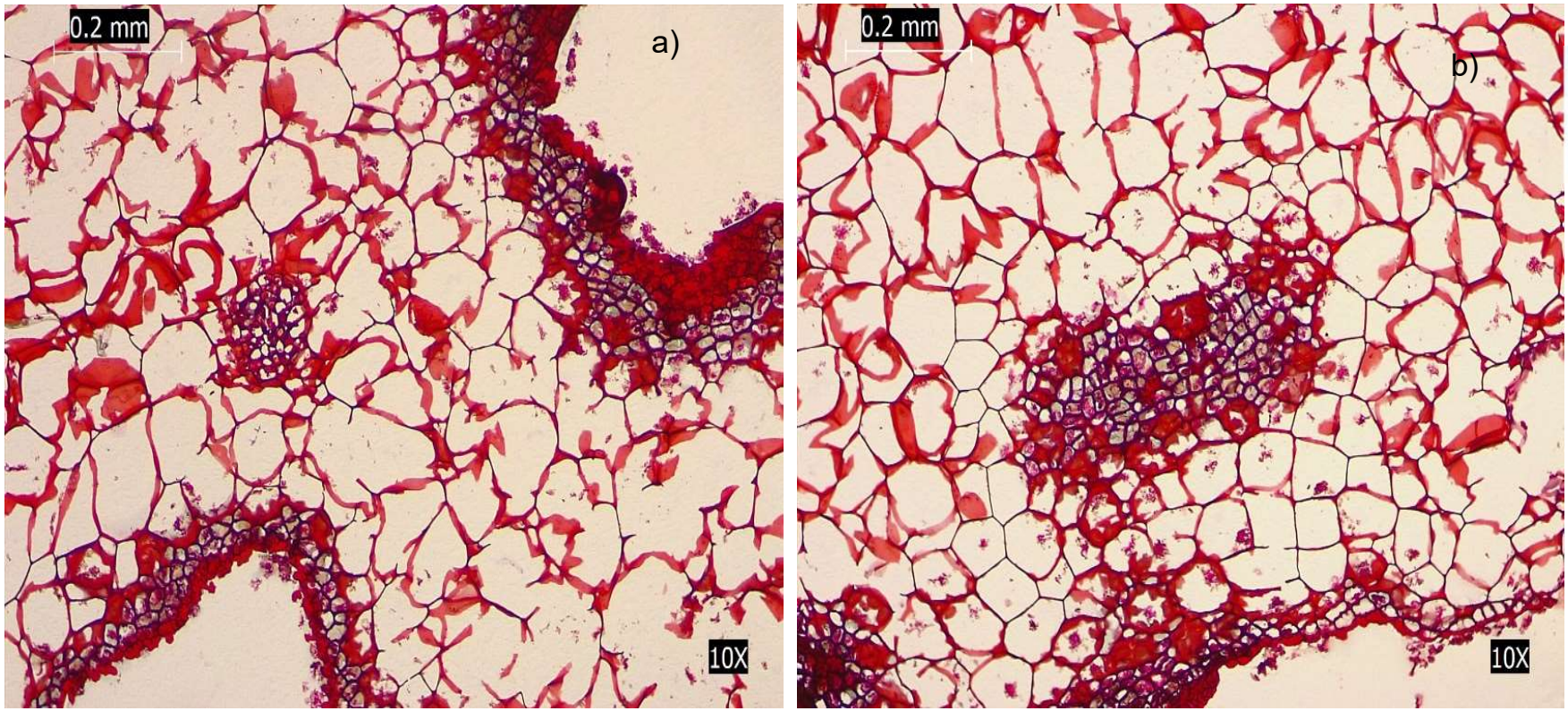


Figure S 1 Histology images of the *Sargassum* species leaf; longitudinal sections stained with Alcian Blue Safarin. No evidence of internal microbial structures were observed. Both a) and b) visualised using X10 magnification with a scale of 0.22mm. b) Focusing on apparent cell structure observed within the leaf

6.2 Chapter 3 Supplementary Information

Table S 4 List of samples, species and associated sequence reads highlighting the quality reads used for taxonomic classification

Sample	Species	No. raw reads	No. cleaned reads	% Removed
Dec, direct contact, #1	*Sargassum,* biofilm	244984	211995	13.47
Dec, direct contact, #2	*Sargassum,* biofilm	171734	151780	11.62
Dec, direct contact, #3	*Sargassum,* biofilm	258038	235231	8.84
Dec, direct contact, #4	*Sargassum,* biofilm	242852	214353	11.74
Dec, direct contact, #5	*Sargassum,* biofilm	305644	267310	12.54
Dec, isolation, #1	*Sargassum,* biofilm	207682	187798	9.57
Dec, isolation, #2	*Sargassum,* biofilm	182506	164902	9.65
Dec, isolation, #3	*Sargassum,* biofilm	190672	173259	9.13
Dec, isolation, #4	*Sargassum,* biofilm	247534	223553	9.69
Dec, isolation, #5	*Sargassum,* biofilm	194716	175746	9.74
Dec, systemic, #1	*Sargassum,* biofilm	225198	206369	8.36
Dec, systemic, #2	*Sargassum,* biofilm	204869	188318	8.08
Dec, systemic, #3	*Sargassum,* biofilm	175638	161323	8.15
Dec, systemic, #4	*Sargassum,* biofilm	209020	179646	14.05
Dec, systemic, #5	*Sargassum,* biofilm	209954	190141	9.44
Feb, direct contact, #1	*Sargassum,* biofilm	216266	192894	10.81
Feb, direct contact, #2	*Sargassum,* biofilm	224649	190947	15.00
Feb, direct contact, #3	*Sargassum,* biofilm	171207	150822	11.90
Feb, direct contact, #4	*Sargassum,* biofilm	279291	244693	12.39
Feb, direct contact, #5	*Sargassum,* biofilm	262592	226961	13.57
Feb, isolation, #1	*Sargassum,* biofilm	240934	209600	13.01
Feb, isolation, #2	*Sargassum,* biofilm	279302	245267	12.19

Feb, isolation, #3	*Sargassum,* biofilm	262760	237739	9.52
Feb, isolation, #4	*Sargassum,* biofilm	178116	156914	11.90
Feb, systemic, #1	*Sargassum,* biofilm	230813	193237	16.28
Feb, systemic, #2	*Sargassum,* biofilm	285096	252618	11.39
Feb, systemic, #3	*Sargassum,* biofilm	246910	219420	11.13
Feb, systemic, #4	*Sargassum,* biofilm	201352	173411	13.88
Feb, systemic, #5	*Sargassum,* biofilm	241012	208787	13.37
May, direct contact, #1	*Sargassum,* biofilm	205345	169368	17.52
May, direct contact, #2	*Sargassum,* biofilm	185980	165846	10.83
May, direct contact, #3	*Sargassum,* biofilm	222103	193574	12.84
May, isolation, #1	*Sargassum,* biofilm	303732	270211	11.04
May, isolation, #2	*Sargassum,* biofilm	232653	207066	10.99
May, isolation, #3	*Sargassum,* biofilm	192670	174528	9.42
May, systemic, #1	*Sargassum,* biofilm	234314	198214	15.41
May, systemic, #2	*Sargassum,* biofilm	261163	229970	11.94
May, systemic, #3	*Sargassum,* biofilm	288051	262464	8.88
Dec, direct contact, #1	*Montipora*	156042	136065	12.80
Dec, direct contact, #2	*Montipora*	141538	120437	14.91
Dec, direct contact, #3	*Montipora*	147976	130299	11.95
Dec, direct contact, #4	*Montipora*	155591	126279	18.84
Dec, direct contact, #5	*Montipora*	119046	95367	19.89
Dec, isolation, #1	*Montipora*	178614	147760	17.27
Dec, isolation, #2	*Montipora*	172996	146590	15.26
Dec, isolation, #3	*Montipora*	248063	210301	15.22
Dec, isolation, #4	*Montipora*	158923	135729	14.59
Dec, isolation, #5	*Montipora*	155273	127986	17.57
Dec, systemic, #1	*Montipora*	179648	135650	24.49

Dec, systemic, #2	*Montipora*	131255	111123	15.34
Dec, systemic, #3	*Montipora*	201949	171269	15.19
Dec, systemic, #4	*Montipora*	134042	112105	16.37
Dec, systemic, #5	*Montipora*	140382	118771	15.39
Feb, direct contact, #1	*Montipora*	82446	54838	33.49
Feb, direct contact, #2	*Montipora*	160264	132982	17.02
Feb, direct contact, #3	*Montipora*	150641	122251	18.85
Feb, direct contact, #4	*Montipora*	219083	184919	15.59
Feb, direct contact, #5	*Montipora*	152461	128789	15.53
Feb, isolation, #1	*Montipora*	154934	132269	14.63
Feb, isolation, #2	*Montipora*	177714	139473	21.52
Feb, isolation, #3	*Montipora*	115370	91584	20.62
Feb, isolation, #4	*Montipora*	187922	159596	15.07
Feb, isolation, #5	*Montipora*	161303	136243	15.54
Feb, systemic, #1	*Montipora*	126280	107462	14.90
Feb, systemic, #2	*Montipora*	227639	198420	12.84
Feb, systemic, #3	*Montipora*	205276	176980	13.78
Feb, systemic, #4	*Montipora*	181638	156208	14.00
Feb, systemic, #5	*Montipora*	146829	115702	21.20
May, direct contact, #1	*Montipora*	197915	160535	18.89
May, direct contact, #2	*Montipora*	222823	187354	15.92
May, direct contact, #3	*Montipora*	226448	193813	14.41
May, direct contact, #4	*Montipora*	240885	195422	18.87
May, direct contact, #5	*Montipora*	206166	172256	16.45
May, isolation, #1	*Montipora*	182888	155217	15.13
May, isolation, #2	*Montipora*	174952	141033	19.39
May, isolation, #3	*Montipora*	139981	118726	15.18

May, isolation, #4	*Montipora*	161604	137570	14.87
May, isolation, #5	*Montipora*	201246	173411	13.83
May, systemic, #1	*Montipora*	172208	144471	16.11
May, systemic, #2	*Montipora*	184212	157449	14.53
May, systemic, #3	*Montipora*	153007	122191	20.14
May, systemic, #4	*Montipora*	129800	110822	14.62
May, systemic, #5	*Montipora*	175920	147156	16.35
Seawater, Dec, #1	n/a	135441	98441	27.32
Seawater, Feb, #1	n/a	186724	161289	13.62
Seawater, Feb, #2	n/a	236191	205012	13.20
Seawater, May, #1	n/a	208766	182645	12.51
Seawater, May, #2	n/a	239915	208668	13.02

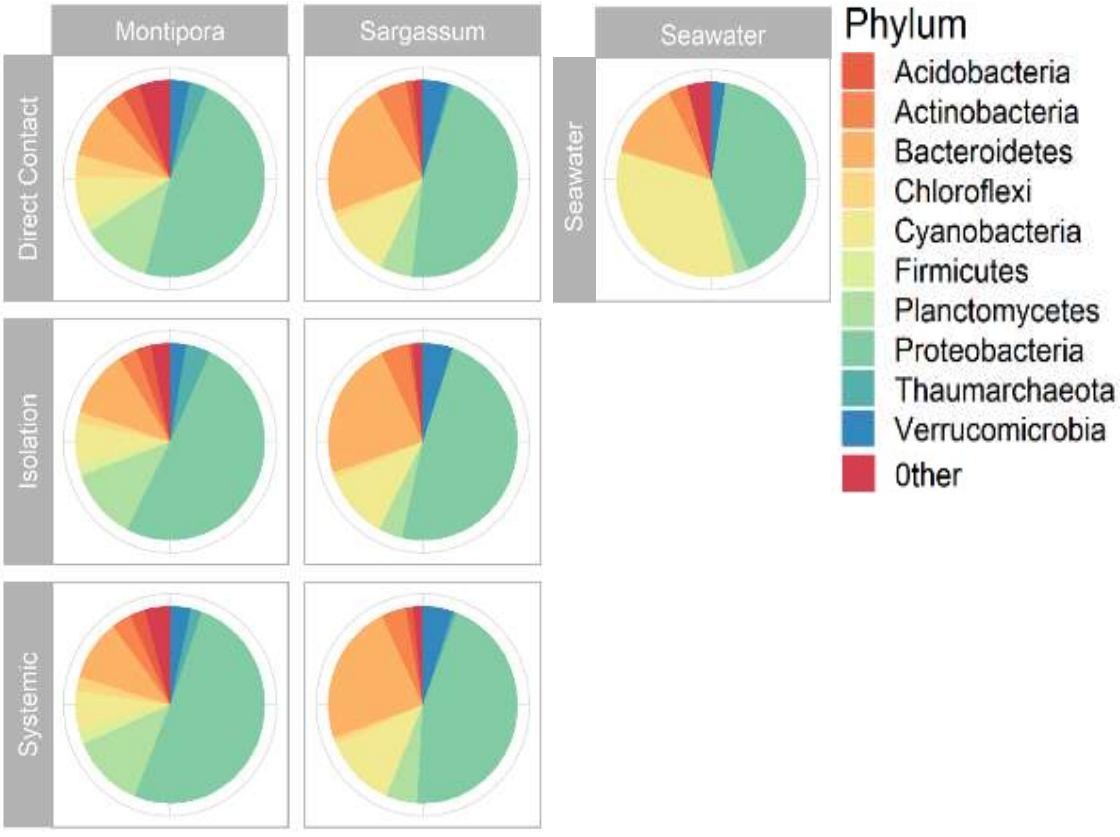


Figure S 2 The most abundant microbial phyla associated with *M. aequituberculata, Sargassum* species and seawater across proximity treatments. "Other" includes phyla representing <1% of all sequences. Relative abundances are percent values total sequences.

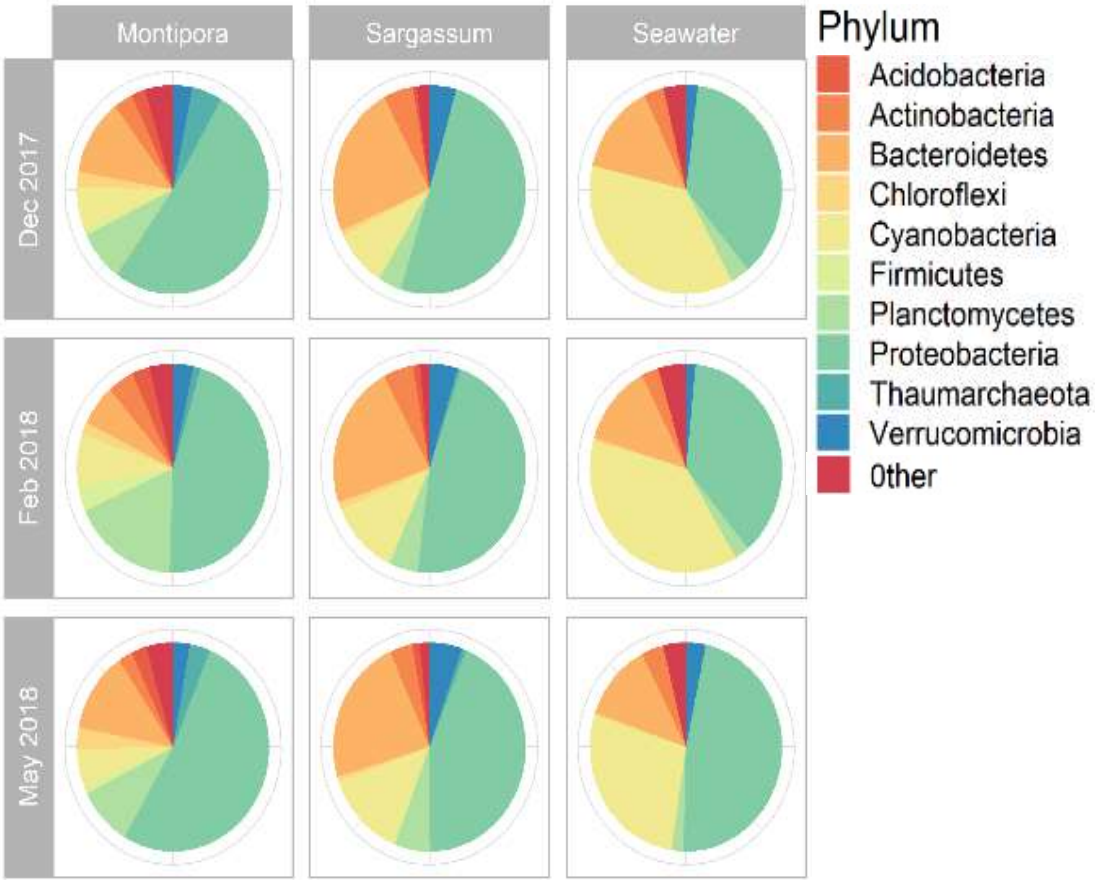


Figure S 3 The most abundant microbial phyla associated with *M. aequituberculata, Sargassum* species and seawater across sampling time points. "Other" includes phyla representing <1% of all sequences. Relative abundances are percent values total sequences.

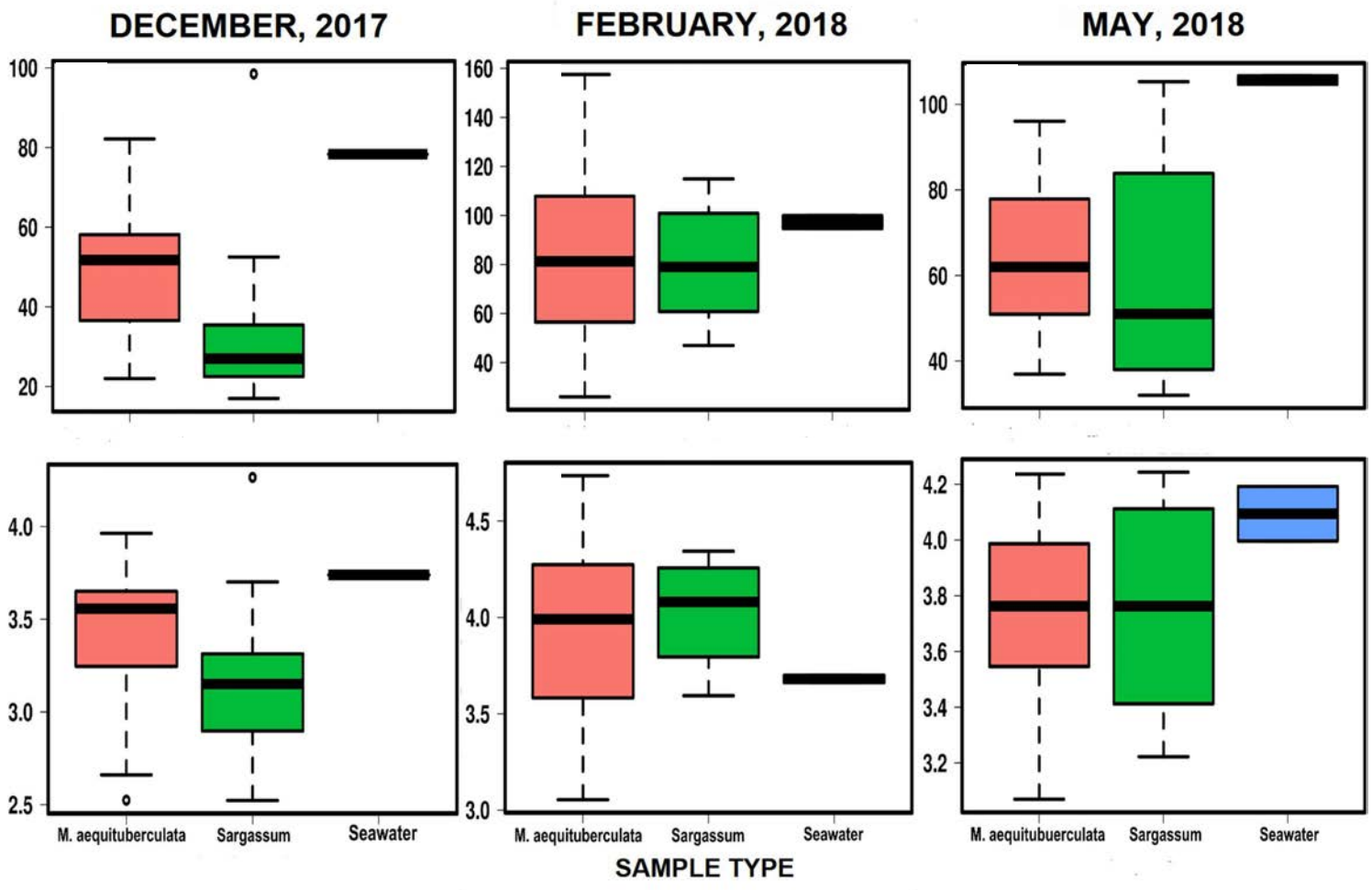
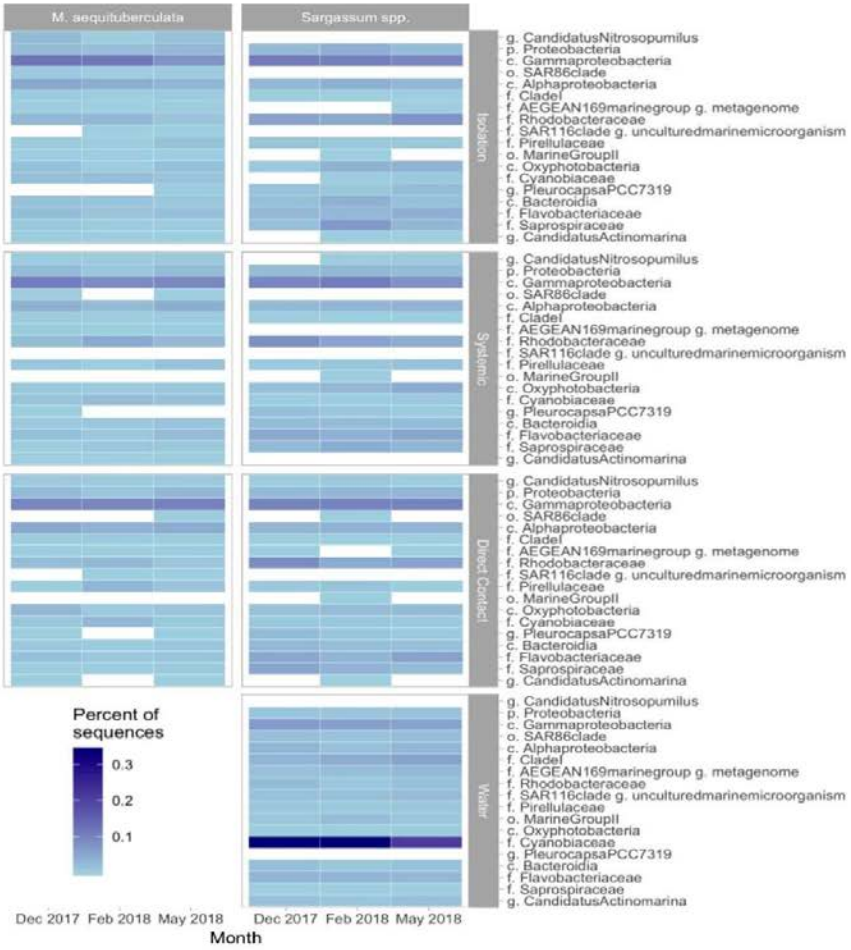


Figure S 4 Alpha diversity parameters highlighting diversity comparisons between *M. aequituberculata, Sargassum* and seawater. Faith's richness: a) December 2017, b) February 2018, c) May 2018. Shannon's diversity: d) December 2017, e) February 2018, f) May 2018



ASV	Source	BLAST ID/Locus	Reference
g. Candidatus nitrosopumilus	Coral	FJ215997	Lins-de-Barros et al., 2010
p. Proteobacteria	Seawater	FJ895215	Siboni et al., 2009
c. Gammaproteobacteria	Seawater	LC138362	Kato and Yamigashi, 2016
o. SAR86clade	Seawater	MH077493	Berube et al., 2018
c. Alphaproteobacteria	Seawater	KY276146	Park and Han, 2016
f. Clade I	Seawater	MH077429	Berube et al., 2018
f. AEGEAN169 marine group	Seawater	MH077099	Berube et al., 2018
f. Rhodobacteraceae	Sediment	KR921057	Bezerra et al., 2015
f. SAR116clade	Seawater	KC425558	Yeo et al., 2013
f. Pirellulaceae	Sediment	MH313195	Pan, Y., 2018
o. Marine Group II	Sponge	DQ299288	Holmes and Blanch, 2007
c. Oxyphotobacteria	Microalgae	AB491627	Chikuni et al., 2009
f. Cyanobiaceae	Seawater	FJ497744	Choi and Noh, 2009
g. Pleurocapsa PCC7319	Biofilm	FJ594841	Siboni et al., 2008
c. Bacteroidia	Macroalgae	KC595533	Twigg et al., 2013
f. Flavobacteriaceae	Coral	MK175937	Goldsmith et al., 2018
f. Saprospiraceae	Biofilm	DQ269087	Longford et al., 2005
g. Candidatus Actinomarina	Seawater	JX405848	Zhang et al., 2012

Figure S 5 ASV analysis of the top 20 more abundant taxon in 100% of samples present across *M. aequituberculata*, *Sargassum* species, seawater and sampling time point. The darker the blue, the most abundant the ASV and where white, the ASV was not present. Corresponding table: BLAST information of each taxa extracted from the NCBI database.

Table S 5 Benthic community investigation at Geoffrey Bay, Magnetic Island. % *M. aequituberculata* and % *Sargassum* estimated by the proportion of the quadrat taken up by each organisms. % Diseased *M. aequituberculata* estimated by the percent of the *M. aequituberculata* plate in each quadrat with signs of disease. Sign of disease is a description of what the disease looked like. % *M. aequituberculata/Sargassum* contact estimated by the percent of *M. aequituberculata* completely smothered by *Sargassum* species within each quadrat. Number of *Sargassum* holdfasts is raw counts of the total number of holdfasts in each quadrat. *Sargassum* canopy height was quantified by measuring the height of three randomly selected *Sargassum* thalli from holdfast to tip and the mean height calculated

Sampling trip	**Quadrat no.**	**% *M. aequituberculata***	**% *Sargassum***	**% Diseased *M. aequituberculata***	**Sign of disease (if present)**	**% *M. aequituberculata*/ *Sargassum* contact**	**No. *Sargassum* holdfasts**	***Sargassum* canopy height (cm)**
Dec 2017	1	70	40	0	N/A	25	22	77
Dec 2017	2	50	35	2	Small black patch	20	5	65
Dec 2017	3	35	15	5	Small black patch	10	10	49
Dec 2017	4	15	55	2	Small black patch	5	16	59
Dec 2017	5	45	30	5	Small black patch	20	7	45
Dec 2017	6	30	20	0	N/A	15	9	41
Feb 2018	1	20	60	0	N/A	20	21	52
Feb 2018	2	15	35	0	N/A	0	12	47
Feb 2018	3	80	20	0	N/A	20	9	42
Feb 2018	4	25	30	0	N/A	5	14	57
Feb 2018	5	25	85	0	N/A	15	12	59
Feb 2018	6	5	30	0	N/A	5	10	38
May 2018	1	15	15	0	N/A	5	11	22

May 2018	2	20	20	0	N/A	5	16	24
May 2018	3	10	5	0	N/A	30	30	30
May 2018	4	10	15	0	N/A	50	20	23
May 2018	5	35	10	0	N/A	5	13	31
May 2018	6	15	20	0	N/A	5	17	27